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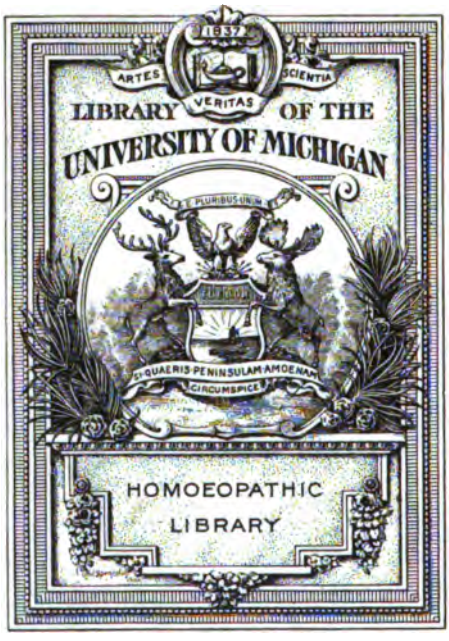
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MODERN URINOLOGY

A SYSTEM OF

URINE ANALYSIS AND DIAGNOSIS.

BY

CLIFFORD MITCHELL, A. B., M. D.
CHICAGO, ILL.

PROFESSOR OF CHEMISTRY, CLINICAL URINOLOGY AND RENAL DISEASES
HAHNEMANN MEDICAL COLLEGE
CHICAGO, ILL.

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PREFACE

The general practitioner does not examine urine as much as he would were it not for certain practical difficulties encountered in making the tests. It is the aim of this book to show in plain language how to overcome these various difficulties, as well as to give the usual information afforded by the numerous authorities. Such information without carefully described particulars is of no avail to the busy doctor, and the author, therefore, strives to supply these much-desired particulars by minuteness of detail. By means of a series of "Laboratory Notes" running through the text the doctor is informed of the many perplexities, difficulties, and fallacies he is likely to encounter in the performance of the tests, and is advised how to overcome them.

The requirements of modern medicine demand an entirely new book, hence the author has not attempted any revision of his older books.

A feature which it is hoped will be appreciated by those who may wish to test their knowledge is a synopsis of the contents of each chapter, printed at the beginning of the chapter. Such synopsis is, in the author's opinion, preferable to the stereotyped list of quiz questions.

A relatively large amount of space has been devoted to the subject of diagnosis by the urine, and it is the aim of the author to provide a large fund of information on this important branch of clinical medicine, suited to the wants not merely of the student of medicine, but of practitioners, surgeons, and even specialists in the various departments.

The author desires to express his obligations and thanks to Dr. G. G. Starkey, of Chicago, for aid in revising the manuscript.

CLIFFORD MITCHELL, M. D.

140 N. State St., Chicago, January 1, 1912.

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MODERN URINOLOGY

CHAPTER I.

THE CHEMICAL COMPOSITION AND PHYSICAL CHARACTERISTICS OF THE URINE.

Chemical composition and analysis of urine.
Physical characteristics of normal urine.
The volume of urine and its variations.
CLINICAL DEFINITIONS.—Polyuria, hydruria, oliguria, anuria, suppression, retention.
Important causes of polyuria.
Important causes of anuria; obstructive and non-obstructive suppression.
Retention of urine.
The urine of different periods in the twenty-four hours.
"Day" urine and "night" urine.
Nocturnal polyuria.
PHYSIOLOGICAL DEFINITIONS.—Urina sanguinis, cibi, potus.
Frequency of urination.
Frequency of urination with pain.
The collection of urine for examination.
The shipping of urine for examination.
Apparatus for collection and for measurement.
American and French measures of volume.

THE URINE.

DEFINITION.—Chemically speaking, urine is water containing in solution about five per cent. of solid substances, of which urea constitutes one-half and common salt one-quarter. Other constituents are nitrogenous, inorganic salts, pigments, etc., large in variety but small in quantity. In addition to the substances in solution there are certain ones in suspension, viz., the contents of the mucous cloud or nubecula.

TABLE I.

COMPOSITION OF THE URINE.

According to W. Simon the average composition of human urine is as follows:

Water			95.760
a. Nitrogenous constituents.	Urea	2.50	2.794
	Uric acid	0.04	
	Creatinine	0.06	
	Hippuric acid	0.04	
	Xanthine bases	0.003	
	Ammonia	0.001	
	Coloring matter, mucus and other proteins	0.15	
b. Non-nitrogenous organic constituents.	Oxalic acid	0.002	0.146
	Phenols and aromatic sulphuric acids	0.024	
	Other organic matters	0.12	
c. Inorganic constituents.	Chlorides, Phosphates, Sulphates of Sodium, Potassium, Magnesium, Iron		1.300

The average normal excretion on a mixed diet is as follows:

Volume, 1200 c.c.
Specific gravity, 1020.
Total solids, 60 grammes (930 grains).

The various solids may be reckoned in amount as follows:

Urea, 30 grammes (460 grains).
Common salt, 15 grammes (230 grains).
Sulphates, 5 grammes (75 grains).
Phosphates, 4 grammes (62 grains).
Ammonia, 0.75 gramme (12 grains).
Urates, 0.50 gramme (8 grains).
Miscellaneous, 3 grammes (46 grains).

On a vegetarian or mostly vegetarian diet these figures are much less.

Physiologically, urine is an excretion from the kidneys, in part filtered from the blood and in part elaborated from waste materials in the blood, which are derived from the foods and tissues. The composition of human urine varies, being influenced by the following circumstances: Water, food, and medicine taken:

season, temperature, humidity, time of day; age and sex; work and exercise; emotions and blood pressure.

Physical Characteristics of Urine.—Normal freshly voided urine is clear yellow, of aromatic odor, saline somewhat bitter taste, and slightly acid reaction. The specific gravity ranges from 1002 to 1030 at different times in the day, but of the 24 hours' mixed urine the range is from 1018 to 1025. Exposed to air urine undergoes decomposition.

The Volume per 24 Hours.—In healthy adults each kidney secretes about 0.5 c.c. per minute, or 60 c.c. (2 fl. oz.) per hour. The urine flows into the bladder in gushes at intervals of from 10 to 30 seconds. The healthy adult voids urine on an average five times in 24 hours, the total volume being from 1200 to 1500 c.c. (40-50 fl. oz.). The normal range in volume, according to age, sex, diet, etc., is as follows:

Adults.—Usual normal range, 900 to 1500 c.c., but 500 to 2,000 not abnormal. Women pass less than men; 800 to 1,000 c.c. not uncommon.

Children.—In infancy 60 c.c. for each kilogramme, or one fluid-ounce per pound; at the end of the first month, average 200 or 300 c.c.; during first year, 300 to 400 c.c.; third to sixth year, 300 to 600 c.c.; eighth to twelfth year, 600 to 900 c.c. (20 to 30 fl. oz.).

Physiological Variations.—The volume of urine is *increased* by cold, humid weather, moderate exercise; in lower altitudes; by hearty eating; by excessive drinking of water, milk, beer, gin, mineral waters; by use of diuretics (citrates, acetates, digitalis, theobromine, calomel, salicylates); by inhalations of oxygen and by electrical stimulus.

The volume of urine is *decreased* by dry hot weather, over-exercise, in high altitudes, and by abstinence from eating and drinking.

Pathological Variations.—The volume of urine is *temporarily increased* by nervous excitement, hysteria and migraine (after paroxysm), constipation, and following diminution of the flow from any cause, as after convulsions, at the height of acute infections, during convalescence, and after reduction of dropsy.

The volume of urine is *intermittently increased* in hydronephrosis due to temporary removal of obstruction to the flow of urine. The volume of urine is *temporarily decreased* by rest and quiet; after administration of anesthetics; in poisoning by a number of drugs (mercury, cantharides, arsenic, carbolic acid, opium); and during convulsions, colics, vomiting, hemorrhages, profuse diarrhea, shock and collapse; in the course of acute renal congestion, acute nephritis, acute exacerbations of chronic nephritis, and acute uremic attacks.

The volume of urine is more or less *permanently increased* in diabetes (mellitus and insipidus), chronic interstitial nephritis and amyloid kidney, renal tuberculosis, multilocular cystic kidney, chronic pyelitis, cardiac hypertrophy with increased blood pressure; in organic nervous diseases, especially of the medulla; and in neurasthenia.

The volume of urine tends to be *decreased persistently* in many chronic diseases, as chronic bronchitis, chronic diarrhea; in chronic lead poisoning; in subacute nephritis with dropsy; in passive congestion of the kidney from any cause; in cardiac dilatation (weak heart and dropsy); in melancholia.

Clinical Definitions.—A large excess of urine per 24 hours is known as

Polyuria may be nocturnal only, as in kidney diseases.

Hydruria denotes urine of low specific gravity, as in hysteria, or after drinking freely of water, etc.

Oliguria denotes the voiding of too little urine; *anuria* the voiding of little or no urine. *Suppression* of urine is due to failure of the renal function; *retention* to inability of the bladder to expel the urine.

Clinically Important Causes of Polyuria.—Diabetes insipidus (enormous quantities of urine), diabetes mellitus, chronic interstitial nephritis; moderate polyuria in chronic pyelitis, and in neurasthenia.

Anuria and Suppression.—Either non-obstructive or obstructive. In non-obstructive cases such urine as is voided is concentrated and high-colored, containing abnormal constituents, casts, blood, etc. In obstructive suppression any urine voided is likely to be pale and watery, without presence of abnormal constituents.

Non-obstructive suppression, partial or complete, may be found in the following cases: After anesthetics, reflexly after surgical operations or procedures upon the genito-urinary tract, internal injuries, during severe hemorrhages, in strangulated hernia and renal colic; in acute nephritis, acute uremic attacks of chronic renal lesions, irritation of the kidneys reflected from lesions of the lower urinary tract (stricture, etc., especially after alcoholic excess); in hysterical or convulsive attacks; thrombosis of the renal vein; in the cold stage of cholera; in severe acute infections.

Terminal anuria may occur in fatal renal lesions and diabetes mellitus.

Obstructive suppression is due to conditions in which the ureters are involved, as in impacted calculus, obstruction from a tumor, valves, or twists.

Retention of urine in the bladder may cause anuria, as in prostatic cases, coma, spasm of the bladder, or when any obstruction to the flow exists. This condition is not uncommon in alcoholic coma or when tight stricture is present. Occasionally it is due to calculus in the urethra or some mechanical obstruction in the vesical neck. Presence of a tumor above the symphysis pubis should always suggest retention, especially in prostatics or in alcoholics.

The Urine at Different Times in the 24 Hours.—The most urine is voided from 2 to 4 p. m., and the least from 2 to 4 a. m. Normally the individual voids twice as much urine when up and about as is formed during the hours when he is in bed. It is convenient to call the former *day urine* and the latter *night urine.*

Nocturnal Polyuria.—A relative excess of night urine occurs in the case of persons who eat and drink during late hours, and in women who are wakeful or "nervous" at night. *Pathologically* it is found in subacute and chronic nephritis, diabetes mellitus, heart diseases, and toxemias, as in constipation. In subacute nephritis with dropsy nearly all the urine is voided during the night. In diabetes mellitus the volume of night urine is nearly or fully equal to the day urine.

Physiological Definitions.—The urine voided on rising in the

morning is termed *urina sanguinis;* that voided after meals, *urina cibi;* after drinking, *urina potus.*

Frequency of Urination.—This may occur either with or without polyuria. With polyuria it occurs in nervous conditions, as sexual neurasthenia, fright or worry; in diabetes and chronic interstitial nephritis. Without polyuria in spinal diseases disturbing the nerve supply to the bladder, and in congestive or inflammatory diseases of the urinary tract; or in acute prostatitis, cystitis, vesiculitis, or prostatic hypertrophy; during renal colic or in consequence of irritation from sand, gravel, stone or hyperacid urine; in irritation from urethral or preputial conditions; in malignant or tubercular diseases of the genito-urinary tract (nocturnal frequency); rectal diseases (reflex irritation), as dysentery or worms; irritation from diapers, sexual impulses, etc.

Frequency of Urination With Pain.—Occurs in diseases of the renal pelvis or of the lower urinary tract; rarely in diseases of the renal parenchyma.

Collection and Measurement of the Urine.—Since the urine varies in constitution at different times in the day, it is necessary to collect and examine the entire volume for 24 hours. The patient should be instructed as follows: On rising in the morning the urine is voided but not collected. Begin collection after breakfast, just before going to stool. All the urine voided from breakfast to bedtime, inclusive, is to be collected in a clean bottle labeled "day urine" and provided with a clean cork. All the urine voided after going to bed and that voided on rising in the morning is collected in a second bottle marked "night urine." When the patient brings or sends the 24 hours' collection it is advisable for him to include also an extra sample as freshly voided as possible. The patient should void urine before going to stool in order to avoid loss, which in many cases may be considerable. Women should, whenever possible, take pains to avoid admixture of vaginal fluids with the freshly voided sample. Patients, during the collection period of 24 hours, should take the customary amount of exercise and eat and drink as usual, but those who habitually drink large quantities of fluid should reduce the quantity to a minimum on account of the danger of dissolving tube

casts in an excess of fluid. The age, weight, and condition of appetite of the patient should be noted by memoranda on the labels of the bottles.

When urine is sent from out of town various circumstances conspire to defeat the purpose of the analyst. In the first place, therefore, some preservative should be added in definite amount to the "extra" sample of urine. Boric acid is the best urine preservative. I am in the habit of furnishing out-of-town patients and physicians with a small envelope containing the proper amount of boric acid preservative necessary to keep three or four ounces of urine indefinitely, so that urine reaches me from all parts of the country in as good condition as when voided, provided the bottle used is clean, the cork clean, and the preservative added as soon as the urine is voided.

Tardy delivery by express companies may be avoided by marking the package "Perishable—rush," and breakage, by "Glass, with care." It is also advisable to calculate the time of arrival of the package so that it may not be received late on Saturday, on Sunday, or on a legal holiday. In all out-of-town cases notice of the shipment of the package should be sent by mail with memoranda of the history and clinical symptoms. When such precautions as to collection, preservation and forwarding are taken, the work of the analyst becomes easier, and the clinical value of his report far greater. All the urine voided in 24 hours is to be shipped, and none thrown away on any account or for any reason. In cases where the diagnosis is obscure the urine of each micturition should be collected in a separate bottle, with the time of urination marked on it. Urine suspected of containing a small amount of bile or blood should be examined as soon as voided.

Apparatus for Collection and Measurement.—The ordinary chamber vessel should not be used. Wide-mouthed bottles, glass fruit jars or any containers which can be scalded out and closed by cap or cover are to be preferred, since some urine undergoes decomposition rapidly on exposure to air. Hospital bed pans should not be used unless odorless and scrupulously clean. Care should be taken to prevent admixture of even a small amount of feces. Persons employed in active business during

the collection may use flat quart or pint bottles which can be carried in inside pockets of coats.

It is well to supply the patients with containers the volume of which is ample to hold all the urine voided in 24 hours, since inexperienced persons are likely to throw away an excess.

The urine of infants may be collected by placing a clean sponge over the genitals, fastening the diaper over it. The sponge should be squeezed out into a clean container.

For measuring urine *graduates* are used. The ordinary graduate with a large base or foot and large neck and lip breaks easily and is not desirable. Neither is the tall cylinder advisable for measuring urine, although for chemical measurements at least one should be provided.

For measuring urine use the graduate without foot or neck sold by Schmidt & Ade, 109 Randolph Street, Chicago (Fig 1).

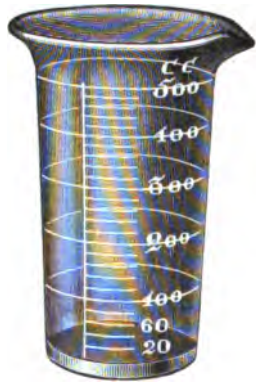


FIG. 1.—Graduate of Form Recommended.

Measures of Volume.—The cubic centimeter, which represents the volume of one gramme of distilled water at 4° C., is the unit of volume. One thousand cubic centimeters are collectively termed one liter.

One liter equals 1.0567 U. S. quart=33.81 fluid ounces.

One minim equals 0.06 c.c.; one fluid drachm equals 3.70 c.c.

One fluid ounce equals 29.57 c.c.; one pint equals 473.12 c.c.

One quart equals 946.24 c.c.; one gallon equals 3785.3 c.c.

Roughly we reckon 30 c.c. equals one fluid ounce except for large quantities of urine, in which case 29½ is preferable. Records of the volume of urine should also be kept in terms of cubic centimeters.

ARITHMETIC CONSTANTS.

1 Centimeter = $^1/_{100}$ meter = 0.3937 inch.
1 Cubic Centimeter = $^1/_{1000}$ liter = 16.23 minims = 0.061 cubic inch — weighs 1 gramme at 4° C.
1 Cubic Inch = 266 minims = 16.386 c.c. — weighs 252.46 grains or 16.372 grammes.
1 Dram (Troy, or Apothecary) = 60 grains = 3.888 grammes.
1 Fluidram = 60 minims = 3.696 cubic centimeters.
1 Fluidounce (imperial) = 28.4 c.c. = 1.7329 cubic inches — weighs 437 $^1/_8$ grains at 62° F.
1 Fluidounce (U. S. wine measure) = 8 drams = 480 minims = 29.57 c.c. = 1.8047 cu. in. — weighs 456 grains or 29.57 grammes.
1 Foot = 12 inches = 144 lines = 0.30479 meter.
1 Gallon (imperial) = 277.27 cubic inches = 4.543 liters — weighs 10 pounds (70,000 grains).
1 Gallon (wine) = 8 pints = 231 cubic inches = 3.785 liters — weighs 8.34 pounds (58,328 grains).
1 Gallon (solid) = 268.8 cubic inches.
1 Grain (Troy) = 0.0648 gramme.
1 Gramme = 15.4323 Troy grains = weight of 1 c.c. of water at 4° C.
1 Inch = 12 lines = 2.54 centimeters.
1 Kilogramme = 1000 grammes = 32.1 Troy ounces = 2.2046 avoirdupois pounds = weight of a liter of water.
1 Liter = a cubic decimeter = 1000 c.c. = 33.8 fluidounces = 1.056 wine quarts = 61.027 cubic inches.
1 Meter = 3.28086 feet = 39.37043 inches = about one forty-millionth of earth's meridian.
1 Millimeter = 1000 micromillimeters = $^1/_{1000}$ meter = 0.0393 inch (about $^1/_{25}$ inch).
1 Minim = 0.0616 c.c. — weighs 0.95 grain.
1 Ounce (Troy) = 480 grains = 31.1 grammes.
1 Ounce (avoirdupois) = 437.5 grains = 28.35 grammes.
1 Pint (imperial) = 20 fluidounces = 567.93 cubic centimeters.
1 Pint (U. S. wine) = 16 fluidounces = 473.179 cubic centimeters.
1 Pound (Troy) = 12 ounces = 5760 grains = 0.37324 kilogramme.
1 Pound (avoirdupois) = 16 ounces = 7000 grains = 0.45359 kilogramme.
1 Quart (imperial) = 40 fluidounces = 69.97 cubic inches = 1.1358 liters.
1 Quart (wine measure) = 32 fluidounces = 58.3 cubic inches = 0.9463 liter. (Hill.)

CHAPTER II.

THE PHYSICAL CHARACTERISTICS OF NORMAL AND OF ABNORMAL URINE: COLOR, APPEARANCE, AND ODOR.

The color of normal urine.
Physiological variations in color; effect of age, sex, exercise, etc.
Pathological variations in color; effects of polyuria, nephritis, diabetes, etc.
Urine which darkens on standing; effect of alkapton, melanin, and of accidental constituents.
Color of cloudy urine; effect of bile, pus, blood, urates, etc.
Color due to accidental constituents; effect of methylene blue, dyes, vegetables, drugs, etc.
Dark hues (carbolic acid, etc.).
Relation of color to disease; effect of jaundice, cystic kidney, melanotic sarcoma, ochronosis, alkaptonuria, carbolic acid poisoning, etc.
Appearance of normal urine.
Physiological variations in appearance; effect of meals, cold, exposure to air, etc.
Pathological variations in appearance; causes of urine cloudy when voided; presence of deposited phosphates, oxalates, urates, uric acid; presence of bile, chyle, blood, pus, mucus, epithelium, etc.
Clinical notes on significance of various appearances of urine.
Diagnostic points with reference to cloudy, light colored urine.
Changes in appearance on standing.
The odor of normal urine.
Physiological variations in odor; effect of sex, acidity, weather, concentration, etc.
The odor of stale urine:

Clinical significance of ammoniacal odor, fecal odor, rotten-egg odor, stale fish odor, sweetish odor, fruity odor, sweetbriar odor, etc.

Odors due to accidental constituents, as asparagus, vegetables, drugs.

Lack of odor in renal diseases, etc.

Normal freshly voided urine is yellowish in color, due to presence of urochrome, and clear in appearance. On standing

exposed to the air it darkens slightly until it becomes of alkaline reaction, after which it is lighter in color again. Its odor is agreeable, being slightly aromatic. Red urines derive their color from urobilin, a derivative of bile pigment, and the yellowish, pinkish or reddish pigment adhering to urate sediments is uroerythrin.

Physiological Variations in Color.—The color is *deficient* in children, nurslings, women; during the day as compared with night; after copious draughts of water and milk; after alcoholic drinks; after moderate exercise with water drinking; in alkaline urine as compared with acid urine. The color is ***higher*** in adults, bottle-fed children and men who drink but little; on rising in the morning; after hearty meals and drinking of strong coffee; after violent exercise and free perspiration; in acid urine.

Pathological Variations in Color.—The color is *decreased* in polyuria. Common clinical causes are diabetes, chronic interstitial nephritis and nervous excitement. Poisoning by duboisin is said to decrease the color. Anemia and wasting diseases have pale urine. The color is ***increased*** in oliguria and non-obstructive anuria. Common clinical causes of increased color are acute infections, congestions, dropsies, chronic hepatic diseases, rheumatism and gout; after anesthetics; in pernicious anemia; in long continued intermittent fevers.

Clinical Notes.—Normal color does not necessarily imply normal urine. In serious renal lesions, and sometimes in diabetes mellitus, the color may be normal.

The color of urines which become turbid on cooling is masked by the deposit of urates which should be cleared by warming in order to judge of the color. Urines cloudy from other causes should be filtered for correct determination of the color.

Color Which Darkens on Standing.—A color yellow or red-yellow when voided but becoming dark on standing, possibly almost ***black*** in time, is due to presence of urobilin, alkapton, melanin or accidental constituents (carbolic acid, salol, guaiacol, aspirin, etc.). The darkening is likely to begin at the surface and gradually extend downward by oxidation. In ochronosis and other rare conditions an undetermined black pigment is sometimes excreted.

The Color of Cloudy Urines.—Properly speaking the true color of urine is the color of the clear filtered urine. Clinically we often describe the color of urine as that of matters suspended in it. For example: *milky urine,* due to pus, phosphates, or fat; in children to amorphous urates; *reddish urine,* due to presence of blood or hematoporphyrin; *smoky urine,* due to hematuria; *pink to porter-colored urine,* due to hemoglobinuria; *smoky brown to black urine,* hematinuria (small hemorrhages); *greenish brown to black urine* with greenish foam, due to bile; *a dirty green color* with bluish scum, due to indigo blue in decomposing alkaline urine or whenever indoxyl is enormously increased (cholera, typhoid fever, spinal diseases).

Colors Due to Accidental Constituents.—These are brighter than the urine tints, or in some way peculiar and foreign in their shades. *A trace of bile* produces a bright yellow color in urine; methylene blue, a dirty-blue green.

Less commonly we find *yellow tints* due to carrots, cascara, chrysophanic acid (orange), eosin, frangula, gamboge, picric acid, picrotoxin, resin, rheum, santonin in acid urine, senna, thallin.

Reddish tints may be caused by aloes, alizarin, analgen, anilin chlorhydrate, aspirin, beets, bilberries in acid urine, cascara, chelidonium, frangula, fuchsin, logwood (in acid urine), madder, magenta, mulberries, rheum in alkaline urine, santonin and senna in alkaline urine, sulphonal and trional.

Violet Tints.—Bilberries in alkaline urine, logwood in alkaline urine.

Eosin in red pencils sucked or chewed by children will cause a yellow color in urine which turns bright red with ammonia or other alkali.

Blue or Green Tints.—Blue or green dye stuffs, especially methylene blue; methyl violet; pyoktanin, indigo, saffron, indoxyl, salicylic acid in large doses.

Dark Hues.—These may be caused by antipyrin, arsenetted hydrogen, carbolic acid, catechol (dark olive green), creosote, cresyl, creolin, the cyanides, gallic acid, hydrochinon, kairin, logwood, lysol, naphthol, naphthalene, phenocoll, potassium chlorate,

pyrogallic acid, quinine, resorcin, salicylic acid, salol, sulphuric acid, tannin, tar, thallin, terpin hydrate, turpentine, uva ursi.

Clinical Note.—Shaking the urine will produce a foam the color of which, if greenish yellow, suggests bilé or urobilin.

Clinical Determination of the Color.—This is done by filtering the urine and comparing with Vogel's color scale. Colors are recorded by the numbers on this scale,—I, II, III, etc., unless due to accidental constituents, when they are described according to their characteristics. For observation of color test tubes should not be used, but beakers or white dishes preferred.

On Vogel's color scale, No. I is pale yellow; II, light yellow; III, yellow; IV, red-yellow; V, yellow-red, and VI, red.

The colors of urine may be described as follows: Pale watery urine as in anemia, diabetes insipidus, and after drinking enormously of fluids, is termed *pale-yellow;* next in depth of color is the light colored urine of women and children and the light urine voided at night in cases of nocturnal polyuria, and that of neurasthenics, termed *light-yellow;* normal urine of adults in quantity around 1200 c.c., 40 fluidounces per 24 hours, is *yellow* in color; the urine normally voided on rising in the morning when a pint or less in amount is *red-yellow;* next is the scanty urine of fevers termed *yellow-red;* finally the highly concentrated urine in congestions of the kidney and in hepatic cirrhosis approaches *red.*

The urine colors should be learned from observation of the urine itself. Vogel's color-scale is not given here because the colors, as usually printed, are far too bright.

Relation of the Color to Diseases.—Dark, almost black urines occur in old cases of jaundice, in profuse hemorrhages,—as in multilocular cystic kidney and malignant tumor of the kidney,—melanotic sarcoma, ochronosis, alkaptonuria, and in poisoning by carbolic acid, etc. In the last four cases the urine darkens on standing.

A case is described by Traumann (*Deutsche Med. Wochenschrift,* April 28, 1910), where urine of grass-green color was passed which changed to blue on standing, due to presence of indigo-blue.

THE APPEARANCE OF URINE.

Urine is either clear or cloudy in appearance. Normal urine appears to be clear, but in strong light is slightly cloudy from mucus suspended in it (nubecula). From excess of mucus the urine of women is more cloudy than that of men.

Physiological Variations in Appearance.—The urine may be cloudy two hours after hearty meals, due to deposit of *earthy phosphates;* soluble in 50 per cent. acetic acid. This sediment is common in those fond of vegetables. Urine clear when voided may become cloudy in a cold room from deposit of *urates* if the urine is somewhat concentrated, as on rising in the morning. Stale urine is always more or less cloudy. Urine voided into an unclean vessel soon becomes cloudy.

Pathological Variations.—Urine *cloudy when voided* is of much clinical interest: such urine may contain bacteria, deposited phosphates, carbonates, urates, oxalate crystals, uric acid crystals, bile, chyle, blood, mucus and epithelium. *Bacteria* in freshly voided urine are rare (bacteriuria) unless associated with pus, etc. Such urine never grows clear on standing. On shaking there is a wave-like motion. On filtering, the urine is still cloudy. The cloudiness may be removed by shaking with talc and repeated filtration.

Deposited phosphates with *carbonates* pervade the whole urine, which clears above on standing, with a light colored flocculent sediment at the bottom, soluble in 50 per cent. acetic acid. The urine is feebly acid, neutral or alkaline. Ammoniacal odor suggests triple phosphate crystals, which sparkle in a strong light, and ammonium urate,—small, dark, thornapple crystals.

Deposited amorphous urates are rare in the warm urine, but occasionally occur in it, as in fevers, capillary bronchitis and pneumonia; more commonly appear after cooling and are completely cleared by warming the urine to 48° C. (120° F.). The urine is acid and the sediment is yellow, pink or red. Common in winter, in diseases of the liver, lungs and digestive organs, acute infections and rheumatism.

Oxalate crystals form a slow-settling light colored sediment like an exaggerated mucous cloud, and easily disturbed by shak-

ing; not soluble in acetic acid but soluble in hydrochloric; urine of any reaction; noticed in fevers and digestive disorders.

Uric acid crystals appear like red pepper grains, stick to the sides and corners of the glass, are heavy and settle quickly, forming a reddish sandy sediment: urine acid; common in urine on standing, in gout, rheumatism, diabetes mellitus, and calculus.

Bile pervades the whole urine, which is greenish or greenish brown with green-yellow foam; common in jaundice.

Chyle gives the urine a milky appearance; not affected by heat or acetic acid, but cleared by shaking with ether. A rare condition, due usually to parasites (filaria).

Blood gives the urine a beef-tea or smoky appearance. The reddish sediment is not dissolved by heat nor by a few drops of acetic acid; common in post-scarlatinal nephritis, growths, stone and tuberculosis.

Pus is heavy, opaque, yellowish white or greenish white; in acid urine settles quickly, leaving the urine clear above it. In alkaline urine it is lumpy, stringy and sticky; common in diseases of the bladder and prostate, abscesses, etc.

Mucus and epithelium form a cloud which hangs in the middle of the fluid, settling at once when acetic acid is added; light, transparent and wavy. Common in women and then of no clinical importance.

Clinical Notes.—A sediment of amorphous phosphates occurring after meals is not important, but the persistent sediment constitutes the condition known as "phosphaturia," occurring in nervous debility or strain, in post-gonorrheal cases, and in indigestion. It should not be confounded with seminal fluid, which is almost colorless.

An abundant sediment of triple phosphate crystals usually means stale urine with ammoniacal decomposition. In freshly voided urine it signifies commonly diseases of the bladder or renal pelvis, as in stone, enlarged prostate, etc.

The diapers of children may show a clay-colored or pinkish deposit due to urates and uric acid, the voiding of which causes pain and straining.

The presence of a sediment in urine is no proof of an excess of

the constituents forming it, but merely indicates a change from normal in the concentration or acidity of the urine.

Diagnostic Point.—Most commonly we find the urine permanently cloudy and light colored when freshly voided in diseases of the lower urinary tract or renal pelvis; rarely in nephritis. Gonorrhea, enlarged prostate, cystitis from various infections, tuberculosis and stone are common causes.

Changes in the Urine on Standing.—In some urines on standing an increase in acidity takes place which results in the deposit of uric acid crystals and a darkening in the color. Later, or in most urines first, fission-fungi having action upon the urea form ammonium carbonate, the urine becomes alkaline and cloudy from presence of bacteria and deposit of phosphates and ammonium urate. The color is then lighter and the odor suggests ammonia. If pus or blood is present, a foul odor is additionally noticed.

THE ODOR OF URINE.

Normal and freshly voided urine has an agreeable odor due to unknown aromatics. The urine of women may have a slightly acrid odor from mucus, especially in the summer. Concentrated high colored urine which is highly acid has a stronger but still aromatic odor, as when voided on rising in the morning. Stale urine may have the acrid odor as above; later an ammoniacal odor (change of urea into ammonium carbonate). If albumin, pus, or blood be present in stale urine the odor is putrid or that of sulphuretted hydrogen, ammonium sulphide or methyl mercaptan.

Clinical Significance.—Freshly voided urine of *ammoniacal odor* (decomposition of urea in the body) occurs in suppurative diseases of the urinary tract, cystitis, cancer of the bladder, stone, ulcers, etc.; most commonly in old men with enlarged prostate or in retention of urine from any cause. The chronic condition is called ammonemia or urinary septicemia.

A *fecal odor*, especially in pus in urine, suggests fistula, abscess between bladder and rectum, or involuntary stool, as when women or feeble persons collect urine. *An odor of rotten eggs* (H_2S) in freshly voided urine points to an abscess in the region of the

intestines draining into the bladder. *An odor of stale fish* occurs in bacteriuria; *a repulsive odor* in some cases of pyelitis. *An odor of blood* in freshly voided urine containing blood suggests hemorrhage from the lower urinary tract.

The presence of a large amount of sugar in the urine may be indicated by a *sweetish or hay-like* smell. Acetone in quantity causes *a fruity odor* like over-ripe apples, which clinically is of importance as indicating a severe case of diabetes mellitus.

The rare constituent cystine imparts *a sweetbriar odor* to the fresh urine, but in decomposing urine the odor is foul, like sulphuretted hydrogen or foul sewer-gas.

Vegetable foods and accidental constituents of the urine may impart their peculiar odors: asafetida, beef extract, copaiba, castor oil, asparagus, cauliflower, garlic, valerian, cabbage, carbolic acid, strong coffee or tea, tobacco, alcoholic drinks, myrtol, onion, parsnips, saffron, sandalwood, santal oil, spices, Tolu balsam, nitro-benzole and santonin are among those noted by various observers as giving their characteristic odors to the urine. Turpentine and terebene cause an odor resembling violets.

A lack of odor in the freshly voided urine is noticeable in certain renal diseases in which the urine is of low specific gravity; also in obstructive retention where cysts are formed.

Clinical Note.—Particular care should be taken not to confound the odor of an unclean receptacle with that of the urine itself. The writer has noticed that many a hospital urinal has, when apparently empty, a peculiarly acrid odor due to decomposing urine previously collected in it and not wholly removed. In taking the odor of urine be sure that it is freshly voided into an odorless receptacle.

CHAPTER III.

THE REACTION AND ACIDITY OF URINE.

The reaction of normal urine to litmus paper.
Effect of concentration, copious drinking, meats, etc.
"Acid fermentation;" changes in reaction on standing.
Alkaline decomposition; reaction of stale urine.
Conversion of urea into ammonium carbonate, etc.
Urine alkaline from fixed alkali and from volatile.
Amphoteric reaction.
Physiological variations in the reaction; effect of age, drinks, diet, exercise, ingestion of drugs, etc.
Pathological variations; effects of diseases, as diabetes, fever, gout, stomach diseases, etc., etc.
Clinical notes on hyperacidity; effect on urethritis, cause of albuminuria, sign of lithiasis, diagnostic point in pulmonary tuberculosis.
Clinical test for reaction, use of litmus paper.
How to preserve litmus paper.
Collection of urine for determination of acidity.
Cause of the apparent acidity of urine.
Difficulties in determining the acidity of urine.
Clinical method for determining acidity.
Benedict's "acid units."
Relation of acidity to arteriosclerosis.
Preparation of decinormal sodium hydroxide solution.
Decinormal sodium hydroxide U. S. P.
Folin's method for determining the total acidity, including diacid phosphates and free organic acids.
Acidity expressed in terms of oxalic acid and of hydrochloric acid.
The chemical balance and set of weights.
Determination of acidity by estimation of sodium dihydrophosphate.

The Reaction of Urine.—Normally the urine is slightly acid in reaction, turning blue litmus paper red. The degree of acidity depends largely upon the concentration, urine of high specific gravity—as on rising in the morning—being more acid than after drinking copiously. In some cases, however, the urine grows less acid after meals, following the acidity of the stomach contents,

reaching its lowest acidity three or four hours after the meal and increasing in acidity again as the food passes into the intestines. This diminution in acidity is known as the "alkaline tide," but has been denied by Benedict, of Buffalo. The cause of the acidity of urine is not definitely known, but is probably due to diacid phosphate of sodium, acid salts of uric acid and free organic acids.

Acid Fermentation.—Some urines on standing show an increase in acidity, usually between six and twelve hours after being voided. The change may be attended by darkening of the color and deposit of uric acid crystals, urates, calcium oxalate, and presence of fungi (penicillium glaucum) and bacteria. Such urines turn blue litmus paper to bright red.

Alkaline Decomposition (Stale Urine).—As already stated, urine on standing becomes alkaline from change of urea into ammonium carbonate (Chapter VI.). This change takes place in from 24 to 48 hours. The warmer the weather the sooner. Ammonio-magnesium phosphate is formed as a double salt from magnesium phosphate and some of the ammonia in the carbonate. On standing quiet such urine acquires a surface coating or film of bacteria, vegetable growth and crystals. Such urine turns red litmus blue. In certain diseases, especially of the bladder, alkaline decomposition takes place within the body.

Fixed Alkali and Volatile Alkali.—Ammoniacal urines have a strong "urinous" or dung-hill odor, and the blue color imparted to litmus soon fades, being due to *volatile alkali,* so-called. Urine alkaline from fixed alkali (carbonates of sodium and potassium) is noticed after meals, has no dung-hill odor and the blue color on litmus is permanent.

Urine alkaline from volatile alkali is more irritating to inflamed mucous surfaces (ulceration or abscess) than that which is alkaline from fixed alkali. The phosphates, however, deposited persistently in urine alkaline from fixed alkali may cause irritation and pain during urination, especially at the end.

Amphoteric Reaction.—Occasionally urine is found which turns the red paper blue and the blue paper red. This is due to the simultaneous presence in the urine of diacid phosphate (acid) and the disodium phosphate (alkaline).

Physiological Variations in the Reaction.—The reaction is *more acid* in the following conditions: after abstinence from drinking, on nitrogenous diet, from circumstances increasing the concentration, as prolonged muscular exercise, free perspiration, and starvation; following the ingestion of mineral acids, acid drinks, benzoic acid, boric acid, and saccharin.

The reaction is *less acid* or alkaline in the following conditions: after copious draughts of liquids, on non-nitrogenous diet, and normally in new-born infants; in cases of polyuria; following the ingestion of vegetable acids and alkaline salts excreted as alkaline carbonates: tartrates, citrates, etc.

Pathological Variations in the Reaction.—The reaction may be *more acid* in hyperchlorhydria unless free fatty acids are present in the urine (lipaciduria); in diabetes mellitus (lipaciduria and nitrogenous diet); in diseases with increased tissue metabolism: fevers, pneumonia, pleurisy, scarlet fever, certain diseases of the liver, sometimes in indicanuria; in scurvy, leukemia, and dermatitis; in gout, rheumatism, lithiasis, and chronic nephritis. The reaction is *less acid* or alkaline in hyperchlorhydria, as after vomiting or washing out the stomach; in polyurias, as diabetes insipidus (but not mellitus); in neurasthenia, nervous debility, nervous exhaustion, anemias, chlorosis, and certain abdominal diseases (typhus, enteritis, flatulence).

Clinical Notes on Hyperacidity.—A highly acid urine is irritating to the mucous membranes of the genitourinary tract, and causes much suffering in urethritis and posterior urethritis. It also especially aggravates any existing disease in women.

Albuminuria and cylindruria are sometimes dependent upon hyperacidity of the urine.

Persistent hyperacidity is frequently a sign of lithiasis, and becomes one of the factors in the diagnosis of renal calculus. In cases where complaint is made of pain just above the symphysis pubis without pus in the urine or other sign of cystitis, the urine is frequently hyperacid. In gouty, rheumatic and lithemic conditions, and in some cases of chronic interstitial nephritis, the urine may be hyperacid. A marked increase of acidity is a clinical feature of diabetes mellitus, due in part to

the presence of lactic acid in the milder cases and to volatile fatty acids (diacetic and oxybutyric) in severer cases (see Ammonia).

Persistence of urinary acidity for many days is said to be a factor in the early diagnosis of pulmonary tuberculosis. The urine of consumptives, if kept from contact with air and dust, stays acid for from twelve days to three months or more, while that of healthy individuals retains its acidity no more than seven days on an average.

Clinical Test for the Reaction.—Test the reaction of urine clinically by means of litmus paper, blue and red, dipping a slip of the paper half its length into the sample. Feebly acid urine turns blue paper a scarcely perceptible red, normally acid urine a plainly visible red, and hyperacid urine a bright red. Neutral urine affects neither blue nor red paper; alkaline urine turns red paper blue. Litmus paper should be bought in sheets. The sheets are cut into slips and the slips are kept in tightly corked bottles, one for the red and one for the blue. In the bottle containing the blue paper should be also kept a few grains of ammonium carbonate, but not enough to cause a strong odor of ammonia.

Red paper soon fades and, in general, the blue paper answers sufficiently for clinical purposes, since alkaline urine will turn it perceptibly bluer, if kept as above described, and neutral urine does not affect its color.

Quantitative Determination of the Apparent Acidity in Urine.—In order to make any clinical use of the determination of the acidity care must be taken in collecting the urine for examination. The whole 24 hours' urine is necessary, and it is always safer and better to separate the day from the night urine, and to keep both samples in well-closed glass receptacles in a cool place. The ordinary chamber vessel must not be used at all, for fear of contamination with bacteria.

The patient, during the collection, should abstain from the use of acids and acid drinks on the one hand, and on the other from alkaline salts, as bicarbonate of sodium, acetate of sodium, etc., or vegetable acids. Nor should he drink to excess of any

liquid, but observe his regular habits. Alkaline mineral waters should be abstained from.

The urine of hospital patients can be examined most advantageously for acidity by immediate determination of the acidity of every freshly voided sample of the urine during the day, as well as that of the entire night urine. In this way the possibility of change from decomposition is reduced to a minimum. Patients living at a distance and desiring a determination of the acidity should collect the 24 hours' urine by voiding the urine of each micturition into a separate bottle or jar containing 20 or 30 drops of chloroform and corking tightly.

The apparent acidity of the urine is due to the presence of sodium dihydrophosphate, so-called diacid sodium phosphate. Urine also contains the disodium hydrophosphate (alkaline phosphate), hence the degree of acidity represents the excess of the former salt over the latter. There are other acid salts in the urine as well, hence the determination of apparent acidity is difficult on account of the variety of acid salts, for each one of which there is a different end-reaction point. Moreover, since the decinormal sodium hydroxide solution used in titration is an alkali, it induces—according to Lieblein—the formation of a calcium phosphate; hence the phosphoric acid is not neutralized in constant proportion. Moreover, phenolphthalein used as an indicator is interfered with materially by the presence of ammonium salts. Hence many authorities deny entirely the clinical value of the determination of the acidity. (See Folin method below).

Clinical Method for Determination of Acidity.—Measure off 25 c.c. of the 24 hours' well preserved urine into a beaker, add to it 50 c.c. of water and four drops of the usual 0.5 per cent. phenolphthalein solution. Mix well and titrate with the decinormal sodium hydroxide solution prepared as below. Run in the soda solution from a Schellbach burette (Fig. 2) until a pink color appears in the urine which resists shaking. The number of c.c. of decinormal soda solution divided by 2.5 and multiplied by 10 indicates the degree or percentage of acidity. Thus if 5 c.c. of the soda solution were required to produce a permanent

pink red in the urine $5 \div 2.5 = 2$, and 2 times $10 = 20$ degrees or per cent. of acidity. The normal range is from 20 degrees to 30 degrees. In order to avoid fractions and to obtain the total acidity for 24 hours in figures which are easy to see when in a column or in number, as in hour-to-hour records, Benedict suggests the arbitrary conception of "acid units" analogous to gramme-molecules. Thus the degrees of acidity of a sample of urine are taken to denote the acidity of 1 c.c. of it and the "total acid units" are obtained by multiplying the degrees of acidity by the number of c.c. in 24 hours. Thus, if there are 1200 c.c. in 24 hours, and if 3.7 c.c. of sodium hydroxide are required to neutralize 10 c.c., the relative acidity is 37 degrees and the total acid units 37 times 1200=44400, which is excessive, 30,000 to 40,000 being regarded as normal.



FIG. 2.—Schellbach Burette with Glass Stop-Cock.

Laboratory Note.—In order to obtain the end-reaction more closely it is better to use 25 c.c. of urine diluted with 50 c.c. of water, rather than 10 c.c. of urine, as recommended by most authors. In such diluted urine a quick eye will readily catch the end-reaction by observing the yellow color which just precedes it. Multiply the amount of soda solution used by 4 to obtain degrees of acidity.

Harrower, of Chicago, has devised a tube known as an acidimeter, by using which the clinical determination is easily made.

For more accurate work, especially in diabetic urines, the Folin method is preferable, as given further on.

Clinical Notes.—The clinical importance of the urinary acidity lies in the early recognition of autotoxemia or acidemia, with its tendency to produce arteriosclerosis. Whenever the urinary acidity is persistently above 40 degrees by the Folin method there is danger of degeneration of the blood vessels. If at the same time the amount of total solids in the urine be comparatively low the condition is more serious.

Decinormal Sodium Hydroxide Solution.—In making the above

determination care is to be taken to provide an accurately prepared decinormal sodium hydroxide solution. This can be had from dealers in chemicals in the larger cities, as, *e. g.*, E. H. Sargent & Co., 125 West Lake Street, Chicago, but the physician should be able, if necessary, to make it himself or to instruct his pharmacist how to make it. A solution is first made of decinormal oxalic acid, using Merck's guaranteed reagent. Place 6.255 grammes of the pure crystallized oxalic acid in a liter flask, dissolve in distilled water and fill up to the mark at a temperature of 25° C. (77° F.). The result is decinormal oxalic acid. It should be made fresh every time when it is necessary to standardize the sodium hydroxide solution, which is done as follows: place 10 c.c. of the decinormal oxalic acid solution in a thoroughly clean flask, add a drop or two of an 0.5 per cent. phenolphthalein solution, shake and add from a Schellbach burette with a glass stopcock, while still shaking, a solution of sodium hydroxide containing about 6 grammes to the liter of distilled water. As soon as a slight permanent pink is obtained cease adding the hydroxide and read off the figure denoting the amount added. Dilute the sodium hydroxide solution as follows: measure the total amount of sodium hydroxide solution on hand, divide it by 10 and multiply the quotient by the excess over 10 of the amount used to obtain the pink color as above, *i. e.*, to neutralize the acid. Then, if it take 12 c.c. of the hydroxide to neutralize 10 c.c. of the oxalic acid and there is 1500 c.c. of the sodium solution made up, then $\frac{1500}{10}$ times 2 equals 300 c.c., which represents the amount of water necessary to add to the 1500 c.c. of the sodium solution in order that 10 c.c. of it may exactly neutralize 10 c.c. of the oxalic acid.

Having diluted the sodium solution titrate the acid again so as to be sure there is no error anywhere; 10 c.c. of the decinormal sodium hydroxide solution should exactly neutralize 10 c.c. of the decinormal oxalic acid solution, shown by the formation of a light permanent pink color in the acid when the last drop of the 10 c.c. of the alkali is added from the burette.

Decinormal Sodium Hydroxide (U. S. P.).—Since it is not always possible to provide a strictly pure oxalic acid, and, more-

over, since it is necessary to make up decinormal solutions of oxalic acid fresh for standardizing purposes, it is well to standardize the decinormal sodium hydroxide according to the directions laid down in the U. S. Pharmacopœia and used by government chemists. Chemically pure potassium bitartrate in amount 0.934 grammes is dissolved in boiling water and titrated with a solution of about 6 grammes of sodium hydroxide in 1,000 of water. Phenolphthalein is added to the tartrate and a part of the soda solution is run in with stirring until the pink color appears, whereupon by calculation the remainder of the soda solution is diluted until 50 c.c. of it will exactly neutralize a solution containing 0.934 grammes of the tartrate. Since the molecular weight of potassium bitartrate is 186.78, it follows that this weight in grammes will exactly neutralize one liter of



FIG. 3.—Erlenmeyer Flask.

normal alkali solution; therefore, 18.678 grammes will neutralize one liter of the decinormal solution, and 0.934 will neutralize 50 c.c., one twentieth of a liter.

Folin's Method for Determination of the Acidity.—Obtain the total acidity, which indicates that of the diacid phosphates and free organic acids, as follows: measure out carefully, using a pipette, 25 c.c. of urine into a 200 c.c. Erlenmeyer flask (Fig. 3), add one or at most two drops of an 0.5 per cent. phenolphthalein solution and 15 to 20 grammes of finely pulverized potassium oxalate. Shake for one minute, then immediately titrate with decinormal sodium hydroxide solution, the shaking being continued. The sodium hydroxide is added until a faint pink color persists on shaking. Calculate percentage and total acidity as above.

Laboratory Notes.—An objection to Folin's method is the foam which is formed on shaking the urine with the oxalate. Hence the author prefers to pour the urine-oxalate mixture from the flask into a shallow dish, when the pink color can be more easily seen by blowing the froth to one side. When the yellow-pink color appears it may sometimes be more readily detected by filtering the mixture through glass wool, thus getting rid of the troublesome froth. Obtain a glass funnel with a bulb in it and insert in the bulb a small pledget of *soft* glass wool. Pour the urine into the funnel and it will run through the glass wool clear of foam. Another drop or two of decinormal soda solution may then be run in and the least tint of pink readily seen.

In the writer's experience it is not at all uncommon to obtain practically the same results with Folin's method as with the ordinary clinical method, and since the clinical point in any case is a high degree of acidity the Folin method need not be used in all cases. But if the ordinary method shows an unusually high degree of acidity, it is well to check it with the Folin method.

Acidity Expressed in Terms of Oxalic Acid.—Each c.c. of decinormal sodium hydroxide solution contains 0.004 grammes of sodium hydroxide and is equivalent to 0.0063 grammes of oxalic acid. To find the total acidity of the urine use the following proportion: 25 : the number of c.c. of soda solution used : : volume of urine per 24 hours in c.c. : x.

That is, multiply the number of c.c. of soda solution used by the number of c.c. of urine in 24 hours, and divide by 25; multiply the quotient by 0.0063.

Example: Urine in 24 hours 1500 c.c., number of c.c. of soda solution 10. 1500 times 10 = 15,000; divided by 25 = 600 c.c. of the decinormal sodium hydroxide necessary to neutralize the entire 24 hours' urine. 600 times 0.0063 = 3.78 grammes of oxalic acid. The normal range in terms of oxalic acid is said to be from 2 to 4 grammes.

Acidity in Terms of Hydrochloric Acid.—The total acidity of urine is sometimes expressed in terms of hydrochloric acid; thus the entire acid of the urine for 24 hours is said to represent a certain amount of hydrochloric acid. Since 1 c.c. of normal

sodium hydroxide represents 0.03618 of normal hydrochloric acid, then 1 c.c. of decinormal sodium hydroxide is the equivalent of 0.003618 grammes of hydrochloric acid, and the amount of decinormal sodium hydroxide solution necessary to neutralize 10 c.c. of urine (*i. e.*, 25 c.c. divided by 2.5) if multiplied by 0.003618 will represent the acidity of 10 c.c. urine in terms of hydrochloric acid, and the product multiplied by the number of c.c. of the 24 hours' urine divided by 10 will represent the acidity of the 24 hours' urine in terms of hydrochloric acid. Thus 10 c.c. of sodium hydroxide are used to neutralize 25 c.c. of urine, if 4 c.c. of sodium hydroxide will neutralize 10 c.c. of this same



FIG. 4.—Chemical Balance.

urine. If the urine amounts to 1200 c.c. in 24 hours, then 0.003618 multiplied by 4, or 0.014472 multiplied by 120 equals 1.73, the total acidity in terms of grammes of hydrochloric acid.

The normal acidity of urine per 24 hours represents from 1.15 to 2.3 grammes of hydrochloric acid.

Laboratory Note.—It goes without saying that for making up the standard solutions, like decinormal sodium hydroxide and others mentioned in this work, an accurate analytical balance and accurate weights are necessary. These can be had of the dealers in chemical apparatus in the large cities, as, for example,

E. H. Sargent & Co., and are also possessed by institutions of learning in the smaller ones. A balance with agate knife edges, made by Becker, of Rotterdam, may be had of E. H. Sargent & Co., which, with the Becker "3d quality" weights, is sufficiently accurate for laboratory work, the whole costing less than $25.00. (Fig. 4.)

Determination of the Acidity by Estimation of the Sodium Dihydrophosphate in Urine.—This is held by some authors to be the best method of determining the acidity. Determine the total phosphoric anhydride (P_2O_5) by the method described in Chapter X. Precipitate from 50 c.c. of urine all the disodium hydrophosphate (Na_2HPO_4) by adding to it a normal solution of barium chloride (122 grammes per liter) in the proportion of about 10 c.c. of the barium solution to each 0.1 gm. of total phosphoric anhydride found in the titration as above. Make up to 100 c.c. with distilled water, filter clear, and titrate the phosphoric anhydride in 50 c.c. of the clear filtrate representing 25 c.c. of urine. Multiply results by 4 to obtain the amount in 100 c.c. of urine. Subtract the result per 100 c.c. of urine found in the second titration from that found in the first, and the result is the amount of phosphoric anhydride existing as sodium dihyprophosphate (NaH_2PO_4). Subtract three per cent. of the result of the second tritation and add it to the result of the last subtraction for correction.

Clinical Note.—Before drawing conclusions as to the clinical significance of the acidity of urine it is always well to calculate the total solids and to compare the quantity of them with the amount of acidity. It stands to reason that a urine of low specific gravity and small amount of total solids should also be low in acidity, hence a high acidity in such urine (like a large amount of indican for example) is of greater pathological significance than where the specific gravity is high and the total solids high.

CHAPTER IV.

THE SPECIFIC GRAVITY AND TOTAL SOLIDS OF URINE. THE CONSISTENCE AND FROTHINESS. THE TEMPERATURE. GASES IN THE URINE.

The specific gravity of normal urine and its range.
Physiological variations in specific gravity; effect of age, time of day, diet, exercise, drugs, etc.
Pathological variations; effect of diabetes, fevers, nephritis, etc.
Relation of the specific gravity to the volume and color in diseases.
Determination of the specific gravity; the urinometer.
Requisites of a good urinometer.
How to take the specific gravity of urine; the author's method.
Precautions to be observed; effect of temperature, etc.
Taking the specific gravity of small volumes of urine.
Taking the specific gravity of cloudy urine.
The total solids in urine; average for twenty-four hours.
Deductions from this average for diet and exercise.
Physiological variations in the quantity of total solids; effect of hearty eating, exercise, drinking, childhood, etc.
Pathological variations; effect of diabetes, infections, nephritis, toxemias, etc.
Clinical note on gynecological cases.
Prognostic points in pregnancy, nephritis and exudative diseases.
Diagnostic hint when solids are low.
Quantitative determination of the total solids; exact method and clinical method by French and American weights.
The total solids not urea; determination and clinical significance.
The consistence of normal urine.
Effect of alkalinity and pus on consistence.
So-called "thick" urine due to urates or sediment.
Causes of slow filtering of urine.
Effect of chyle.
The foam of urine; effect of albumin, bile and sugar.
The temperature of urine; determination; the use of the chemical thermometer.
Comparison of Centigrade with Fahrenheit degrees.
Gases in urine; proportion and kinds.
Pneumaturia; causes and significance.

The specific gravity of urine depends upon the percentage of solids dissolved in it. Normally the specific gravity ranges from 1002 to 1030 at different times in the day, and from 1018 to 1025 in the mixed urine of 24 hours. In infants the specific gravity may be 1005 or lower. Pathologically it may go as high as 1060.

Physiological Variations in Specific Gravity.—The specific gravity is *higher* in adult life, on rising in the morning, after copious perspiration, and muscular exercise; on animal diet or diet more rich in salts; following the administration of soluble salts (acetates, citrates, etc.).

The specific gravity is *lower* in infancy; after being chilled; in cool weather; during rest; on diet poor in salts, as milk; after drinking freely, especially of alcoholic liquors.

Pathological Variations.—The specific gravity is *higher* in diabetes mellitus, in oliguria and anuria (non-obstructive); in fevers, acute infections; in congestions, acute and chronic; after general anæsthesia; in colics; when there is increasing dropsy; in melancholia, "oxaluria," lithemic conditions, certain hepatic diseases, and in some cases of neurasthenia; occasionally in essential albuminuria and in any nephritis with great excess of albumin in the urine.

The specific gravity is *lower* in diabetes insipidus, in polyurias, reduction of dropsy, convalescence from acute diseases, various toxemias; in nervous excitement, anæmia, chlorosis, hysteria, chronic wasting diseases; before the onset of uremic convulsions (not puerperal); at the fatal termination of acute diseases; in any nephritis without much albumin in the urine.

RELATION OF SPECIFIC GRAVITY TO VOLUME AND COLOR.

Volume diminished, color increased, specific gravity increased, in fevers, dropsy, oxaluria, renal congestions, lithemia and certain hepatic diseases; cerebral and gastric neurasthenias, colics, spasmodic conditions; pain; preceding convulsions.

Volume increased, color diminished, specific gravity decreased, in convalescence, reduction of dropsy, diabetes insipidus, chronic interstitial nephritis, amyloid kidney; nervous polyuria, hysteria after the crisis; after relief from colics, spasmodic conditions and pain; after recovery from convulsions.

Volume increased, color decreased, specific gravity increased: in diabetes mellitus only.

Volume decreased, color decreased, specific gravity decreased: in some cases of subacute nephritis, in anemias—except pernicious,—in debility, especially of women and children, during acute uremic attacks, in subacute and chronic nephritis, in the last stages of chronic interstitial nephritis.

Volume diminished, color increased, specific gravity diminished: in pernicious anemia (increase of iron).

☞ **Determination of the Specific Gravity.**—Obtain a urinometer of the Squibb type (Fig. 5), which has a *round* stem, carefully made with accurate division of the fractions of degrees, which gradually approach one another the nearer they are to the bulb, allowance being properly made for the weight of the stem above the liquid. The figures on the stem should read 1,000, 1,010, 1020, 1030, etc., and between them on the line designating 1005, 1015, 1025, etc., is *a plainly visible dot* which saves time in the reading, as it enables the eye to catch quickly the line which denotes the intervening five. The instrument should be standardized at 25° C. or 77° F., which figures should be printed on it. In other words, it should sink to the mark 1000 when tested in distilled water at 25° C. It should be provided with a pasteboard container bearing a label on the outside on which is to be read the statement of the test by the makers for variations in the readings according to temperatures, and the corrections for the same. In taking the specific gravity of urine use the 24 hours' quantity whenever it is to be had, since the specific gravity of urine varies greatly according to the hour, the food, the liquids taken, exercise, temperature, etc., etc. For example, after drinking heartily the specific gravity may be normally as low as 1005, while on rising in the morning it may normally be as high as 1030. Take, then, the specific gravity of the whole 24 hours' urine in one of the graduates shown in Figure 1, if there is urine sufficient to float the urinometer. The advantage of this method is twofold: the urinometer, being small in comparison with the bulk of the urine, does not either cling to the sides of the glass nor raise the urine by capillarity so high above the actual level

of the fluid as when a small fluted jar or cylinder is used. Hence it is desirable never to use the cylinder supplied by dealers except for small quantities of urine.

In taking the specific gravity he sure that the eye is on a level with the surface of the liquid, and be sure to make allowance for the curve of capillarity rising above the actual surface of the liquid. Thus, if the apparent level of the liquid is 1014, careful observation will show that the real specific gravity is nearer 1015, capillarity raising the surface of the liquid up to the line 1014.

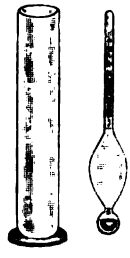


FIG. 5.—Urinometer and Jar.

☞ The advantage of the author's method of the determination of the specific gravity of the whole 24 hours' urine in a large graduate can be readily proved by taking the specific gravity, first as above and then in the small cylinder provided by dealers. In case, however, there is not enough urine for the graduate, the small cylinder may be used as follows: obtain one, if possible, which has no lip but is entirely round at the top. In any case, fill the cylinder nearly full, so that when the urinometer is floated in it the liquid shall rise *just to the edges* of it, but not so high that the surface is noticeably curved. Then, with the eye exactly on a level with the surface, take the reading and allow at least one degree for capillarity. Urinometer glasses, each with a wide foot, may be used. These hold more than the ordinary cylinder and should be filled about three-fourths full of urine. In taking the specific gravity remove all foam by blowing it off or by use of filter paper, dry the urinometer by wiping with a cloth before using, and on lowering it gently into the liquid give it a slight spin to prevent adhesion to the side of

the vessel. Finally wait before reading until the urinometer comes to rest; then, after the reading, force the urinometer down, allow it to rise again and repeat the reading. It will frequently be noticed that a reading too quickly taken varies from one after the instrument is completely at rest. If the temperature of the urine is much different from the standard of the urinometer, 25° C. (77° F.), either warm or cool the urine to this temperature, using a chemical thermometer, or else add or subtract according to the following rule: for every three degrees Centigrade above the temperature at which the urinometer is standardized add one to the reading. Thus suppose the temperature of the urine is 28° C., suppose the urinometer is standardized at 25° C., and suppose the reading to be 1018, the real specific gravity at 25° C. is 1018 plus 1, or 1019. The urine being warmer than 25° C. shows a lower specific gravity at 28° C. than it would at 25° C. Again, if the temperature of the urine is below that at which the urinometer is standardized, subtract one for every three degrees Centigrade of difference. Thus, suppose the temperature of the urine is 22° C., and suppose the urinometer is standardized at 25° C., and suppose the reading be 1021; subtract 1 from 1021 and we have 1020, the true reading at 25° C., since the urine being colder than 25° C. the specific gravity is higher than at 25° C. In Fahrenheit degrees add or subtract 1 for every 5.4 degrees.

When the amount of urine is too small in the jar or cylinder for the urinometer to float in it, it is well not to attempt to take the specific gravity at all. Quantitative determination of urea, requiring only 1 c.c. of urine, and of the chlorides, requiring only 5 c.c., will enable the clinician to judge of the amount of solids present sufficiently well without taking the specific gravity.

There is, however, a small instrument to be had, devised by G. A. DeSantos Saxe, which is handy for use in cases where urine obtained by ureteral catheterization, voided by infants or emergency patients, is to be examined. Such an instrument is known as the urine pyknometer and is graduated in the reverse order as compared with the urinometer, *i. e.*, 1060 at the top of the stem and 1,000 at the bottom.

Laboratory Note.—Care should be taken to take the specific gravity in urine which is clear, in order that the allowance for capillarity can be distinctly seen. This is not so important, however, when the author's method is followed, *i. e.*, that of taking the specific gravity of the entire 24 hours' quantity in a large graduate.

Cloudy urine may be made clear by filtering through three filter papers folded together. (Filter papers about 4 inches in diameter are serviceable for clinical work, and should be of the variety known as "rapid." E. H. Sargent's No. 500 answers the requirements.)

THE TOTAL SOLIDS IN URINE.

The amount of total solids in the urine of the healthy person is influenced by the age, weight, height, diet and exercise, bearing a certain relation to the specific gravity. The average, however, for those between 20 and 40 years of age on a mixed diet and taking ordinary exercise is *one gramme per kilogramme of weight* (15.432 grains for 2.2 pounds = seven grains per pound). Between 40 and 50, there is a reduction of 10 per cent. from this total for 24 hours, between 50 and 60 of 20 per cent., between 60 and 70 of 30 per cent., over 70 of 50 per cent.

Furthermore, certain additional deductions from the average must be made for diet and exercise; deduct 5 per cent. from the theoretical average if the person is in the house, 10 per cent. if in bed, 10 per cent. for not eating the usual amount, 15 per cent. for slight eating and 33 per cent. for fasting ten or more days.

Example: a person 25 years of age, weighing 75 kilogrammes, on a mixed diet should void 75 grammes of total solids per 24 hours; if he were 45 years of age 75 minus 7.5 = 67.5 grammes; if he were 45 years old and in the house, not eating the usual amount, the 5 + 10 = 15 per cent. must be taken off from 67.5 grammes; that is, 67.50 minus 10.13 = 57.37 grammes.

Physiological Variations in the Quantity of Total Solids.—The total solids are *larger in amount* in adults who eat heartily, exercise vigorously and drink copiously; also in cases where mineral waters rich in salts are used and where soluble salts, as ace-

tates, are administered. They are *smaller* in amount in the case of children (though *more* in proportion to *weight*); in adults who lead a sedentary life, who eat little and abstain from fluids; in persons on a vegetarian or a milk diet.

Pathological Variations.—The total solids are *increased* in diabetes mellitus and insipidus; in diseases with great tissue destruction, as acute infections, especially pneumonia and typhoid fever; sometimes in convalescence from acute diseases.

The total solids are *decreased* in chronic diseases of the kidneys (except when polyuria is marked, as in chronic interstitial nephritis) in renal "insufficiency;" in acute infections (between the time of increase of solids and convalescence); in chronic gynecological conditions and various pelvic disturbances; in cutaneous eruptions, intestinal autotoxemias, anemias, etc. (from inability to take or to assimilate food).

Clinical Notes.—In gynecological cases, "renal insufficiency"—*i. e.*, a low figure of total solids, is often observed and eliminative treatment should always be tried before indiscriminate operative procedures.

Women who pass less than 20 grammes (310 grains) in 24 hours are liable to bronchitis, neuralgia, pleurisy or perimetritis on taking cold. Nervous irritability, cutaneous eruptions, headaches, backaches and various pelvic disturbances suggest a determination of the quantity of solids.

Prognostic Points.—In pregnancy a substantial amount of solids in the urine, according to the age and weight, is a good sign, indicating usually less danger from toxemia.

In chronic renal diseases, when the amount of total solids is already low, a further decrease is an unfavorable sign, often preceding an acute uremic attack.

In exudative diseases (pneumonia) an increase in the amount of solids following a progressive decrease is usually a good prognostic sign, indicating that eliminative treatment is unnecessary.

Diagnostic Hint.—In any case in which the solids are low for the age, weight, etc., a thorough examination for presence of renal disease is advisable.

Quantitative Determination of Total Solids.—The only exact

method is to allow the urine to evaporate spontaneously in vacuo over sulphuric acid. Pour 5 c.c. of urine into a small shallow dish previously weighed, add a drop or two of 20 per cent. acetic acid and let dry until there is no longer loss of weight. An accurate balance and careful weighing are necessary.

☞ **Clinical Determination of the Solids.**—Estimation is made by use of the coefficient of Long (2.6) or of Haeser (2.33). That of Long is to be preferred for urinometers standardized at 25° C. The rule: multiply the last two figures of the corrected specific gravity by 2.6 and this product by the number of liters of urine in 24 hours. The final product is grammes of solids in 24 hours. Example: 1200 c.c. of urine, specific gravity 1006; 6 times 2.6 = 15.6, and 15.6 times 1.2 (liters) = 18.72 grammes solids in 24 hours. Patient is a woman 30 years old, weighing 50 kilogrammes; she should, therefore, pass 50 grammes of solids, hence her excretion, 18.72, is extremely low and requires explanation.

Clinical Determination in American Weights.—The total solids in American weights may be roughly calculated by assuming the last two figures of the specific gravity of the 24 hours' urine to represent grains per ounce of total solids. Thus a specific gravity of 1030 in a urine whose volume was 30 fluidounces per 24 hours would represent 30 grains of solids per fluidounce, or 30 × 30 = 900 grains total per 24 hours. Adding one-tenth of the result obtained to itself makes the figure more accurate, so that in the above example add 90 to 900 for greater accuracy. The figure can be converted to grammes by dividing by 15.432, and the amount obtained compared with the theoretical average of one gramme per kilo of weight, or seven grains per pound weight.

The Total Solids Not Urea.—The total solids having been calculated by the coefficient of Long or of Haeser and the specific gravity as above, an approximate determination of the total salts or solids not urea may be made by determination of the total urea (Chapter VI.) and then subtraction of the figure obtained from that of the total solids. Suppose, for example, the total solids in 24 hours are 57 grammes, then 57 minues 30 = 72 grammes of total salts. The amount of total salts ranges from 20

to 30 grammes per 24 hours, of which sodium chloride is usually about one-half if the person is on a mixed diet. The proportion of salts is, of course, greatly increased by the ingestion or soluble salts, mineral waters, etc.

Clinical Significance.—The usual proportion of urea to solids-not-urea is 0.75 to 1. A decrease in the ratio may signify causes unfavorably affecting the formation of urea, as deficient oxidation, hepatic and renal insufficiency. (See Urea.)

THE CONSISTENCY AND FROTHINESS OF URINE.

The consistence of normal urine is practically the same as that of water. If the urine is alkaline in reaction and at the same time contains mucus and pus, as in chronic cystitis, it becomes viscid and stringy; chyle thickens the urine and fibrin also. Concentrated urine depositing a sediment of amorphous urates is commonly called "thick,' but if such urine be filtered it will be found to be of the usual consistence.

Urine which *filters slowly* through one thickness of filter paper is abnormal and may contain either mucus in excess, pus, or blood. A jelly like mass in the urine on standing should suggest the presence of chyle or fibrin, or that chloroform has been added and is emulsionized with the deposit of the urine. The fibrin coagulum is grayish and insoluble in water, but readily dissolved by the pepsin-hydrochloric acid mixture. It may occur in villous growths of the bladder. In chyluria with fibrinuria the urine may be of a pink color and the coagulation of fibrin may take place within the body. Normal urine foams when shaken, but if such urine be poured into a shallow dish the foam soon subsides.

This is not the case, however, with urine containing albumin, in which the foam is persistent even when but little albumin is present. Sugar in the urine increases the frothiness, and especially after fermentation has set in. Bile increases the frothiness and the foam is greenish-yellow. To a less extent frothiness is increased in urines of high specific gravity, or deficient in acidity, as also when excess of mucus is present. The foam in concentrated urines may be persistent.

THE TEMPERATURE OF THE URINE. GASES IN THE URINE.

The temperature of the urine, when the same as that of the body, serves a purpose in helping to retain the urates in solution, as is evidenced by a copious clay-colored or reddish sediment of these substances when some urines cool on standing. It is well to note the temperature of the urine before taking the specific gravity, and for this purpose a chemical thermometer is useful. Reckon 25° Centigrade equals 77° Fahrenheit, and 37° Centigrade equals 98.6° Fahrenheit. The usual room temperature in winter is 21° Centigrade, = 70° Fahrenheit, nearly. As most urinometers now sold are standardized at 25° Centigrade, it is well to take the temperature of the urine and warm or cool it to this degree before taking the specific gravity. Some urinometers are standardized at 60° Fahrenheit, = 16° Centigrade, nearly. When such are used calculate the total solids with Haeser's coefficient, 2.33, and not with Long's.

Gases in the Urine.—The urine contains normally certain gases in solution in proportion of 16 per cent. by the volume, chiefly carbon dioxide (8 per cent.) with nitrogen (11 per cent.) and a little oxygen (1 per cent.). In rare cases the freshly voided urine bubbles from the presence of gases, as when retention has existed in diabetes mellitus and the retained urine has fermented in the bladder. The condition is known as pneumaturia, and is found in patients who have used the catheter or sound, with the introduction of gas-forming bacteria, or when some vesico-intestinal fistula is present. Vesical irrigation or cystoscopic examination in the knee-chest position may mechanically lead to the entrance of gases into the bladder. The passage of gas is usually at the end of urination and may take place with a loud sound. If the patient is made to urinate with the penis under water the escape of the gases is attended by the rising of bubbles. The same condition is noted when the distal end of the catheter is placed under water. The gases are usually odorless and in composition are probably carbon dioxide and nitrogen, but one of the author's patients claimed to pass a gas in the urine having an odor which suggested sulphuretted hydrogen.

CHAPTER V.

THE PHYSICAL EXAMINATION OF URINE.

The osmotic pressure of urine.
Cryoscopy.
The freezing point of normal urine.
Determination of the freezing point.
The Beckman apparatus.
Clinical significance of variations in the freezing point.
Various coefficients:—Strauss's, Koranyi's, etc.
B. A. McBurney's observations in a case of tubercular kidney.
Electrical conductivity.
Method of Kohlrausch.
Clinical significance of the conductivity.
Refraction of the urine.
The index of refraction.
The quotidian valence of refraction.
"Abnormal waste" and refraction.
Specific volume of the urinary molecule.
Superficial tension.
Stalagmometry and the stalagmometer.
The capillary constant.
The viscidity of urine.
Other physical properties: vapor tension, specific heat, etc.

OSMOTIC PRESSURE.

The osmotic pressure of the urine is determined by cryoscopy, *i. e.*, by observation of the freezing point, since the latter depends upon the total number of molecules of solid matter dissolved in the fluid. The freezing point of pure water being taken as 0° C. (32° F.) that of the urine usually varies between — 1.3° C. and — 2.3° C. (29.66° F. and 27.86° F.). The freezing point is indicated by the Greek delta, Δ. The freezing point varies considerably with the diet, being lower on a diet both rich in salts and poor in liquid, and higher following copious ingestion of fluids. For purposes of diagnosis in renal lesions it is customary to determine the freezing point of the urine from each kidney obtained by ureteral catheterization, but it is probably

better to use the 24 hours' mixed urine collected in a perfectly clean vessel.

DETERMINATION OF THE FREEZING POINT.

The apparatus, an extremely delicate Beckman thermometer graduated in hundredths of a degree, the bulb of which dips into a test tube filled with the urine (Fig. 6). The thermometer is held in place by a cork, so that the mercury bulb is immersed in the fluid under examination, but does not come in contact with any glass surface. Outside of the test-tube is another larger test-tube, either designed as an air jacket or containing

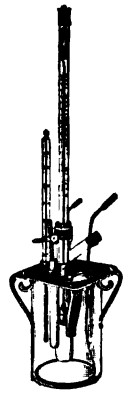


FIG. 6.—Beckmann Freezing Apparatus for Cryoscopy.

alcohol or a solution of glycerine, so that the urine is inclosed in a chamber with double walls having one of these fluids in the interspace. The outer test-tube is surrounded by an ice-salt mixture packed in a strong battery jar or beaker. Rock-salt and ice in the proportions of 1 :3 may be used as a freezing mixture. An essential part of the apparatus is a spiral of platinum wire for stirring the urine constantly so as to keep the temperature of all parts of the urine uniform. There is also provided a larger stirrer for the manipulation of the freezing mixture. In making a determination proceed as follows: Place the freezing mixture in the battery jar and add water if necessary to secure a temperature not lower than 3° C. (37.4° F.). Introduce the fluid to be tested into the inner test-tube; place the thermometer

and platinum stirrer in position and insert the tube into the second larger tube, which has already been inserted through the metal cover of the battery jar. The two stirrers are so manipulated as to insure an equalization of temperature, while the mercury column is closely watched. At first it falls gradually, after which is a sudden rise and the column becomes stationary at a certain point, which point is the freezing point. If the liquid does not freeze a small piece of ice is introduced into it by means of a side tube, which causes instantaneous freezing. Certain precautions must be carefully observed; before and after every determination, the zero of the thermometer must be determined for distilled water. The second zero will be slightly lower than the first and is the zero of the experiment. The stirring should be perfectly rhythmical and with certain instruments automatic stirrers are provided.

The performance of the test unless careful leads to error, and in general the value of the operation is subject to much discussion, other methods of determining the renal function being preferred by modern diagnosticians. Conjoint cryoscopy of the urine and blood give the best results, but the large amount of blood necessary for this work renders it impracticable for general use. A urine freezing above — 1° C. is usually considered abnormal, the total number of molecules of solids excreted being diminished, hence the freezing point rises. In severe cases of chronic nephritis with uremia the freezing point should theoretically be close to or actually at zero. Unfortunately, however, an abnormal freezing point may occur in healthy persons and a normal freezing point can be found in renal lesions. Various coefficients and formulas have been suggested with the hope of obtaining something practical in the diagnosis of renal insufficiency, as for example the quotidian cryoscopic valence of Strauss, Δ times the urine of 24 hours in c.c. (normal 1500-2500), the coefficient of Koranyi, Δ divided by the sodium chloride, normal 1.7 per 100, etc., etc. The principal use of cryoscopy is to determine the functionating power of the other kidney when it is the purpose to remove one. Unfortunately, however, it is claimed that the healthy kidney may be unfavorably

affected by the presence of the other diseased one, and this deceives the cryoscopist. This claim has to a certain extent been verified by the experience of Dr. B. A. McBurney, of Chicago, who, after removing a tubercular kidney from a young woman who was not excreting a great amount of urine, found to his surprise that she excreted a much greater volume of urine from the one remaining kidney than previously from both combined, the amount exceeding 300 c.c. (100 fl. oz.) in 24 hours.

For further details in regard to cryoscopy the reader is referred to the larger books. The method does not appear at present to warrant extended consideration in a work of this kind.

It is, however, possible that in the diagnosis of autointoxication cryoscopy may assume a certain importance.

ELECTRICAL CONDUCTIVITY.

The freezing point of urine as stated above depends on the total number of molecules of solids in solution, whereas the electrical conductivity is dependent upon the total inorganic molecules or ions present. By electrical conductivity is meant the reciprocal of the resistance which a certain amount of a solution between two platinum electrodes of given size and given distance apart offers to the passage of a current of known strength. It is really, then, a measure of the number of electrolytes in solution, that is, of the dissociated ions.

The method of Kohlrausch is usually employed, by which an alternating current is passed between platinum electrodes through the urine. The resistance is balanced on a wheatstone bridge against a rheostat, and the point of equilibrium is determined by a telephone.

The results of observations relating to electrical conductivity, though few, appear to show that normally the value falls below $K = 0.03$, and that in cases of autointoxication the conductivity is generally greater than in health.

REFRACTION OF THE URINE.

It is claimed by Amann and Combe that the measure of the index of refraction of the urine is capable of furnishing to the

clinician findings which are most useful in diagnosis, especially with reference to autointoxication.

The index of refraction may be conveniently determined by the immersion refractometer of Zeiss.

The quotidian valence of refraction is the term given to the product of the number of c.c. of urine in 24 hours measured by the difference in the index of the urine and of distilled water. This valence may be deemed the measure of the ordinary depuration, that is, of the removal of waste matters from the blood. According to Combe this valence should normally be above 10; in intestinal autointoxication readings lower than 8 and sometimes below 2 have been found. Even in cases of polyuria the valence of refraction may show insufficient excretion of solids. It is claimed that in the urine there is an *"abnormal waste"* which escapes chemical analysis, representing in the normal state about 10 parts to 100 of the total solids recognized by analysis; that in diseased conditions this abnormal waste may rise to from 20-40 per 100 of the total waste.

In this nutritional waste the presence of large molecules (which, as a rule, are toxic molecules) is a true indication of defective organic function.

The specific volume of urinary molecules may be measured by the molecular refraction. The ratio $\frac{\Delta n}{\Delta d}$ formed by the difference in the indices of urine and water, divided by that of the density of the two liquids, gives precise information upon the specific refraction and the mean specific volume of the urinary molecules.

SUPERFICIAL TENSION.

According to Amann the lowering of the capillary constant may furnish useful data upon the presence and proportion in the urine of certain abnormal constituents which frequently escape chemical analysis but which are readily detected because of the property they possess of lowering to a greater or less extent the superficial tension of the urine. The measure of superficial tension is undertaken by the drop method, (stalagmometry). The greater the superficial tension of a liquid the heavier will be its

drops before falling, and the smaller will be the number of drops necessary to empty a given volume. That is, the superficial tension measured by stalagmometry depends on the nature of the molecules and not on the molecular weight. The researches of Amann show that the inorganic salts in urine elevate the superficial tension, *i. e.*, diminish the number of drops: urea, sugar, and albumin act indifferently; uric and hippuric acids, fatty acids, aromatics, etc., depress the superficial tension, *i. e.*, increase the number of drops.

By use of Amann's stalagmometer it has been found that in the normal state the number of urinary drops less 100 is half the number represented by the decimals of the specific gravity at 15° C. That is, the normal capillary constant is 0.5. In intestinal autointoxications the capillary constant is no longer 0.5, but rises from 0.8 to as high as 1.5. The stalagmometer delivers 100 drops of distilled water between two marks above and below a spherical expansion. In normal urine of specific gravity 1020 at 15° C. the number of drops, if 110, is, less 100, half the number represented by the decimals of the specific gravity (1020 minus 1000 = 20 and 110 — 100 = 10). In urine from a patient with intestinal autointoxication of specific gravity 1018 at 15° C. the number of drops was 128.

In this case 1018 minus 1000=18, and 128 minus 100=28, and 28 divided by 18=1.56 as the capillary constant.

The viscidity of urine has been studied by Combe and Amann. In autointoxication the viscidity is generally higher than normal.

The vapor tension, specific heat, diffusion, and compressibility have not been sufficiently studied as yet to deserve consideration.

CHAPTER VI.

NITROGENOUS NORMAL CONSTITUENTS OF URINE:— NITROGEN, UREA.

The nitrogenous physiological constituents of urine.
The total nitrogen of urine.
Nitrogen equilibrium.
Nitrogen balance.
Nitrogen partition.
Physiological variations in the total nitrogen.
Pathological variations in the total nitrogen.
Per cent. of nitrogen in the ordinary articles of diet.
Preparation of the patient for nitrogen determinations.
Method of collecting urine for nitrogen determinations.
The Kjeldahl process of determining nitrogen.
Chemistry of urea:—formula, nitrogen content, properties.
Relation of urea to ammonium carbonate.
Microscopical appearances.
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Physiological variations in quantity.
Pathological variations in quantity; effect of pneumonia, typhoid, diabetes, nephritis, etc.
Clinical notes on pneumonia, diabetes, typhoid, ovarian diseases, etc.
Diagnostic points from quantity of urea.
Therapeutic hint as to administration of quinine.
Surgical notes (Chislett and Deaver).
Influence of drugs on excretion of urea.
Tests for urea:—biuret, furfurol, nitric acid.
Isolation of urea.
Quantitative determination:—methods of Moerner-Sjöqvist, Moerner-Sjöqvist-Folin.
Clinical determination—method of Doremus.
Practical use of Doremus' ureometer fully explained.
Calculation of results, examples for practice.
Principle of the hypobromite method, with equation.
Solutions used and how to prepare them; bromine and its dangers.
The handling of bromine and of caustic soda; how accomplished.
How to avoid weighing caustic soda.
How to make the hypobromite.
Use of the nipple pipette.
Technique of the Doremus ureometer and of the Hinds'.
Precautions to be observed.

The **nitrogenous physiological constituents** of urine are urea, the purine bodies (uric acid and purine bases), ammonia, creatine, creatinine, nucleinic acid, allantoin, oxaluric acid, diamines, certain amino acids and peptides. Hippuric acid also contains nitrogen, but is usually classified under the heading of aromatics.

THE TOTAL NITROGEN OF URINE.

The total urinary nitrogen per 24 hours ranges from 10 to 16 grammes. From 83 to 93 per cent. of it is excreted as urea. The sources are the nitrogenous foods and tissue change. In general the waste products of the urine are in part dependent upon the protein contents of the food and, in part only, dependent on the size and condition of the body. The former is a very variable quantity, while the latter is relatively constant.

Nitrogen Equilibrium.—When the amount of nitrogen found in the urine equals the amount of nitrogen taken in the food the person is said to be in a state of "nitrogen equilibrium." The term "nitrogen balance" is used to indicate the proportion between the intake and output of nitrogen. The body in health accommodates the nitrogen output of a given day to the intake of the day before, hence the necessity for determining the total urinary nitrogen for a period of several days in order to obtain an average. A healthy adult can maintain nitrogen equilibrium on an intake of 0.6 grammes of albumin per 24 hours for each kilogramme of body weight, that is, about 5 grains per pound.

The Nitrogen Partition.—Considering the four substances, ammonia, urea, uric acid and creatinine, we find the following percentage distribution according to various analyses (Long):

	1	2	3	4	5	6
Nitrogen in ammonia	4.60	4.78	5.41	5.78	3.88	3.16
Nitrogen in urea . . .	89.68	88.65	89.306	91.62	92 14	92.11
Nitrogen in uric acid	1.44	1.71	1.90	1.72	1.72	1.97
Nitrogen in creatinine	4.28	4.86	3.39	0.88	2 26	2.76
	100.00	100.00	100.00	100.00	100.00	100.00

In addition to the four another urine body, known as oxyproteic acid, may be present to the extent of 3 to 4 grammes daily, or

from 2 to 3 per cent. of the total nitrogen, which if included would modify the above percentages somewhat.

Physiological Variations in the Total Nitrogen.—The total nitrogen is **larger** in amount under circumstances which increase the assimilation of protein: *hearty eating,* meat diet, exercise, hot baths, water drinking; after child-birth. It is *smaller* when the person is a *light eater,* or is on a vegetarian or milk diet, or is of sedentary habits; also during pregnancy.

Pathological Variations.—The amount is *increased* in *acute infections;* in poisoning by arsenic, phosphorus, antimony and metallic compounds; after absorption of exudates and transudates, as in reduction of dropsy; in oxygen starvation, as dyspnea, suffocation, severe hemorrhages; in diseases associated with marked emaciation, malnutrition, or **excessive toxic metabolism; diabetes mellitus,** malignant growths, chronic infections, pernicious anemia, leukemias, scurvy, exophthalmic goitre.

The amount is **decreased** in convalescence from acute diseases, by anything which seriously affects vitality, during formation of exudates and transudates, as ascites, by loss of water, as by sweating, vomiting, and diarrhea. In acute yellow atrophy of the liver, in myxedema, **nephritis,** and in certain hepatic diseases.

Clinical Note.—In nephritis it may be sometimes necessary to determine the nitrogen in the feces, since the gastro-intestinal tract may become a factor in removal of it. The sweat may also contain it in increased quantity.

Per Cent. of Nitrogen in Foods.—The following shows the per cent. of nitrogen in the ordinary articles of diet: uncooked beef, ham, mutton, fowls, fish, 20 per cent.; bacon, 12; cheese, 25; eggs without shells, 6.5; cream, 3; milk, 3; butter, 1; bread, 9 per cent.; wheat flour, 12; rice, 8; oatmeal, 16; potatoes, 2.

DETERMINATION OF THE TOTAL NITROGEN.

Much care is necessary in determining the total nitrogen, not only in the chemical analysis, but in the diet and general control of the patient, so that clinically the determination is seldom made. Determinations of nitrogen must extend over a period of several days and the patient should be placed beforehand in

a state of nitrogen-equilibrium. The amount of nitrogen ingested may be calculated by the aid of tables showing the nitrogen content of various foods.

For ordinary clinical purposes, we proceed as follows: the urine should be collected according to the author's usual directions, *viz.*, from after breakfast on the first day until before breakfast on the second day; the patient is to drink the usual amount of water *but not to flush the kidneys by too copious draughts.* The amount of exercise taken should be usual, not excessive, and the collections should be made while the patient is in his ordinary condition of life and not during some period of unusual circumstances. The diet should be as nearly as possible the usual one and the amount of nitrogen intake calculated by reference to tables of nitrogen.

The urine passed should be collected in sterilized receptacles, kept closed from the air, and in a cool place. The chemical analysis should be made promptly as soon as each 24 hours' collection is finished.

☞ **The Kjeldahl Method.**—The principle of the determination of the total nitrogen of urine is the constant oxidation of it by oxidizing agents into ammonia. This ammonia is in turn converted into ammonium sulphate by sulphuric acid. After this, free ammonia, liberated by the action of caustic soda on ammonium sulphate, is distilled into standard acid, and 1 c.c. decinormal sulphuric acid represents 0.001404 gramme nitrogen. The author advises the technique of Webster, of Chicago, who has made a large number of nitrogen determinations in urine, as follows: 5 c.c. of urine are poured into an 800 c.c. flask and 10 c.c. of sulphuric acid with 1 gramme of cupric sulphate added. The mixture is heated over a low flame until white fumes of the acid appear. Then 5 grammes of potassium sulphate are added and the heat increased for from 30 to 40 minutes, at the end of which time a clear blue green solution is obtained. The operator must beware o fthe fumes, hence a hood is absolutely necessary, Wash down the carbon from the sides by shaking the vessel, taking great care not to lose any of the liquid. Let cool completely. Do not transfer to a second flask. Wash the material

from the neck into the flask and add water to measure 250 c.c. Then add a little talc to prevent "bumping," and 50 c.c. of a 40 per cent. sodium hydroxide solution for every 10 c.c. of sulphuric acid used, in such a way as not to touch the upper part of the neck of the flask. Shake the flask and connect it with a Fresenius' bulb; the outlet tube passes into an Erlenmeyer flask containing 50 c.c. of decinormal sulphuric acid. Rapidly connect the flask to the bulb and condenser. Heat the distilling flask slowly and increase the heat after boiling is regular. Distill for from 20 to 30 minutes or until 150 c.c. have gone over, and continue the distilling until moist red litmus is not affected at outlet tube. Then disconnect the flask from the bulb; wash the condenser tube with acid; disconnect the outlet tube and wash internally and externally with acid.

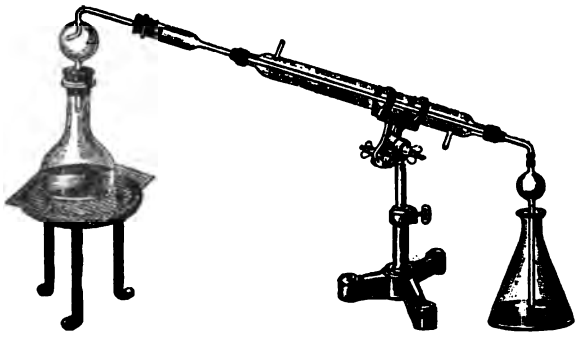


FIG. 7.—Kjeldahl Apparatus modified by Reitmar and Stutzer.

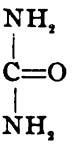
Titrate the acid solution with decinormal sodium hydroxide, using rosolic acid as an indicator. Subtract the amount of alkali necessary from 50 (no. of c.c. of acid in the flask) and each c.c. of remainder corresponds to 0.001404 gramme nitrogen in 5 c.c. of urine.

There are various forms of apparatus sold for the determination of the total nitrogen. Figure 7 shows a Kjeldahl distilling apparatus for nitrogen determination modified by Reitmar and Stutzer and sold at a comparatively low price. E. H. Sargent & Co. sell this apparatus.

Balance experiments should be conducted to determine the

amount of nitrogen, if any, contained in the chemicals used (sodium hydroxide).

UREA.



Chemistry.—Urea, carbamide, CH_4N_2O, the amide of carbonic acid, the principal end-product of the metabolism of proteins, and, therefore, the principal nitrogenous constituent of urine, contains more than 45 per cent. of nitrogen and forms from 85 to 95 per cent. of the total nitrogen in urine. It is the chief vehicle by which the nitrogen of food leaves the body. About 90 per cent. of the nitrogen taken in the food is excreted as urea. When pure it crystallizes in long, colorless, four- or six-sided anhydrous rhombic prisms, melting at 132° C. (238° F.), soluble in water or alcohol, insoluble in ether or chloroform. Urea forms crystalline compounds with certain acids, as nitric and oxalic; it also combines with mercuric nitrate in variable proportions, with various salts, as sodium chloride and chlorides of the heavy metals, forming combinations for the most part crystallizable. It is decomposed by hypobromites with evolution of nitrogen. The crystals of urea contain no water of crystallization, but under the influence of a ferment, as, *e. g.*, the micrococcus ureæ, they take up water, forming ammonium carbonate, according to the equation:

$$\underset{\text{Urea}}{CH_4N_2O} + \underset{\text{Water}}{2H_2O} = \underset{\text{Ammonium carbonate}}{(NH_4)_2CO_3}$$

The ammoniacal odor of decomposed urine is due to this change in urea.

Urea, owing to its ready solubility in water, never occurs in the sediment of urine.

Microscopical Appearances.—Urea itself: Silky, four-sided prisms with oblique ends, or, rapidly crystallized, in delicate white needles. Nitrate of urea: Thin rhombic or hexagonal crystals,

overlapping tiles, colorless plates, whose point has an angle of 82° (Fig. 8). Larger and thicker rhombic pillars or plates are obtained on slowly crystallizing. Oxalate of urea: Rhombic or six-sided prisms or plates, more regular than the nitrate.

Physiology.—Urea is formed by union in the liver of carbon dioxide with amino-groups (leucin) from the nitrogenous food stuffs, and is a product also of the retrograde metamorphosis of the tissues and blood. About 14 per cent. of the total potential energy of the food is said to go to waste in urea. It is probable that the spleen, lymphatic glands and secreting glands aid in the formation of it.

The quantity in urine varies from 1 to 3 per cent., averaging about 2 per cent.; hence 20 to 30 grammes per 24 hours in adults,

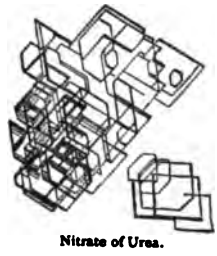


FIG. 8.—Crystals of Urea Nitrate.

averaging 0.4 gramme per kilogramme of weight or about three grains to the pound; less in old age and in fat persons. When the chlorides are normal in quantity urea constitutes not quite half the amount of the total solids. Children void from one gramme per kilo. (3-6 years) to 0.8 gramme (8-11 years) and 0.4 to 0.6 gramme (13-16 years). New-born infants excrete 0.23 gramme of urea per kilo. (reckon 1 gramme per kilo = 8 grains per pound).

Physiological Variation in Amount.—Urea is **increased** by circumstances promoting metabolism: *hearty eating, especially of meat,* mental or physical exercise, hot baths, electrical stimulation. Temporarily by ingestion of excessive quantities of fluids. The most urea is voided six hours after meals and the least in urine of the early morning hours.

In 31 cases examined by the author 29 averaged 2.5 grammes more urea in the day urine than in the night. The other two passed more at night but were in ill health. Alkaline diuretics and drugs which stimulate hepatic function increase urea.

Urea is **decreased** by circumstances which diminish the assimilation of proteins; fasting, **light eating,** vegetarian diet, sedentary habits; in some cases by milk diet; continued ingestion of excessive quantities of liquids. The least urea is voided during the night. Menstruation and pregnancy decrease it; as also drugs which interfere with hepatic functions and certain diuretics.

Pathological Variations in Quantity.—Urea is **increased** by circumstances which favor metabolism of tissue; acute febrile processes (**pneumonia, typhoid,** intermittents before the chill); **diabetes mellitus** and insipidus; certain nervous diseases (progressive muscular atrophy, minor chorea, epileptic attacks, hysterical convulsions, paralysis agitans (?); severe diseases of the blood; malaria, pyemia, dyspneic conditions, severe leukocythemia, scurvy, moderately in pernicious anemia (not always); conditions where exudates or transudates are being absorbed; acute nephritis after absorption of dropsy. Occasionally in the polyuria of chronic interstitial nephritis; in chronic gout, certain gastrointestinal diseases, atrophy from dyspepsia in children, diffused bronchial catarrh without fever; poisoning by belladonna and phosphorus.

Urea is **decreased** by circumstances interferring with metabolism or nutrition: *degenerative changes in the liver* (acute yellow atrophy, cirrhosis, carcinoma, acute febrile jaundice); after the crisis of acute febrile diseases and during convalescence; **in many chronic diseases,** especially nervous diseases and those associated with pain; before death in all diseases; in tumors of the uterus and ovaries; in conditions where exudates or transudates are forming; **acute or subacute nephritis** with increasing dropsy; **chronic interstitial nephritis in the last stage**; by circumstances favoring vicarious excretion (cholera, yellow fever, and diseases attended by excessive vomiting, sweats and diarrhea); in conditions in which the kidneys are unable to excrete the urea formed;

nephritis (subacute or advanced chronic); renal congestion; **before uremic attacks.**

Clinical Notes on Increase of Urea.—In pneumonia if the urea is diminished after the first few days of the disease the case is likely to be one of delayed convalescence. In acute febrile conditions the excretion of urea during the early stages may reach as high a figure as 85 grammes per 24 hours. One of the author's cases (pneumonia) voided 48 grammes of urea on the second day, but averaged only thirty grammes during convalescence. In diabetes a high figure of urea usually attends the periods when the patient is losing in weight, being derived from tissue change. In patients who are stationary or gaining in weight the urea figures are not excessive. In 42 diabetics examined by the writer some years ago 6 passed more than 50 grammes of urea for 24 hours. As high as 130 grammes in 24 hours is claimed by some writers in diabetes mellitus and insipidus. In hectic exacerbations the amount of urea exceeds the normal during the fever.

In typhoid it is said that the excretion may reach 60 grammes for 24 hours or more.

Clinical Notes on Decreased Urea.—In ovarian diseases, especially cancer, the amount of urea is low, sometimes below 15 grammes, or as low as 6 to 8 grammes per 24 hours.

In women who eat little or are anemic, especially if they are nervous or worried, the excretion of urea may fall to 10 grammes per 24 hours, or even lower. In such cases a low figure of urea (below ten grammes) may be noticed during pregnancy without "uremic" conditions necessarily occurring.

Before death from advanced kidney diseases the decrease in urea is marked, and a glistening, greasy coating of urea crystals may be found on the skin, as in the temporal region, on the neck, in the axilla and groin. Preceding acute uremic attacks in chronic renal diseases there is almost always a marked decrease in the amount of urine, total solids, and urea per 24 hours.

In diseases of the liver the decrease in urea is more marked than that of the total nitrogen, an increased ammonia excretion

being noted (See "Ammonia"). Study of the ratio of urea to ammonia is, therefore, valuable in hepatic diseases. In various morbid conditions an aggravation of symptoms may be noticed coincident with a diminution in the urea excretion and subsiding more or less when the latter is increased. C. L. Greene insists that there is a strong possibility of a purely functional insufficiency of the kidneys associated with retention of urea, symptomatically indistinguishable from the uremia of nephritis, but more amenable to treatment.

Before death from acute febrile diseases urea may almost disappear from the urine. The author has observed in one instance a moderate decrease in the amount of urea in paralysis agitans, a disease in which urea is said to be increased.

Diagnostic Points.—It follows from the above that the occurrence of a small excretion of urea per 24 hours is not in itself an infallible sign of uremic poisoning.

On the other hand, in an adult of 70 kilogrammes (150 pounds nearly) eating three meals daily and taking a moderate amount of exercise, less than 20 grammes (310 grains) of urea per 24 hours is an unusually low excretion and demands explanation. If no physiological cause for it can be found, the urine is to be repeatedly examined for albumin and casts, the blood pressure taken, and the heart and eyes examined for evidence of chronic interstitial nephritis.

In cases where leucin and tyrosin are found in the urine and urea is small in amount or almost absent, the condition is likely to be one of acute yellow atrophy of the liver.

Therapeutic Hint.—Repeated determinations of urea in intermittent fever will often foretell by an increase the onset of a chill. But the determinations must be made several times daily, since the rise in urea may be observed only two hours before the chill in quotidian cases, and six or eight hours before in tertian. These determinations are of value in fixing the proper time for the administration of the remedy.

Influence of Drugs in Excretion of Urea.—It is claimed, though sometimes disputed, that quinine, alcohol, iron, lead, mercury, theobromine, digitalis, tea, and valerian, decrease urea.

The author has proved by analysis that diuretin may increase the urinary water greatly but at the same time decrease urea.

On the other hand, it is said that urea is increased by ammonium salts, benzoic acid, coffee, caffeine, colchicum, euonymine, juniper, lithium benzoate, carbonate, and citrate, malt, mineral acids, mercuric chloride, morphine, pepsin, potassium chlorate and chloride, salicylic acid, sodium chloride, squill, and thyroid extract.

To determine the actual value of these statements more accurate observations, made with scientific precision without interference from diet or other disturbing factors, are necessary.

Surgical Note.—Dr. H. R. Chislett, of Chicago, has cautioned the profession about operating when the quantity of urea per 24 hours is below 12 grammes; and more recently Dr. J. B. Deaver, of Philadelphia, has emphasized the risk of prostatic operations in cases where the urine is of poor quality.

TESTS FOR UREA.

Crystals of urea having been obtained, may be tested for solubility, melting point, and crystalline form in the usual manner. The commonest chemical tests are the biuret and furfurol tests.

The Biuret Test.—Fuse about 0.3 gm. (5 grains) of urea in a test tube. Now boil the liquefied crystals till a white residue appears; add about 10 c.c. (2 to 3 fluidrachms) of distilled water; shake thoroughly and add several cubic centimeters (1 to 2 fluidrachms) of a strong solution of sodium hydrate (10 gm. in 25 c.c. water, or 155 grains to the fluidounce).

Set the tube aside for the present. Now make a solution of cupric sulphate in distilled water, strength 0.5 gramme (3 grains) to 100 c.c. (3½ fluid-ounces). Add several drops of this copper solution, drop by drop, to the alkaline solution above made, and a beautiful red-violet color appears at the top. Too much or too strong copper solution gives a blue color with the alkali, which can be seen by adding a large number of drops of the copper solution.

In this test urea is converted into biuret, or allophanamide, a derivative of urea.

The Furfurol Test.—Shake up one c.c. of furfurol with about 15 c.c. of water. To a crystal or two of urea on a porcelain surface add a drop of the furfurol solution and immediately a drop of strong hydrochloric acid, and observe the color change, in which a purplish tint is prominent. Furfurol is an expensive article and waste should be avoided. (Allantoin also responds to the furfurol test.)

Clinical Tests.—In the urine urea may be demonstrated as follows:

1. To a drop of urine, preferably of high specific gravity, on a glass slide, add a drop of nitric acid; warm it gently and cautiously over an alcohol lamp until it is slowly evaporated. Characteristic crystals of urea nitrate may be seen under the microscope even with a low power, 150 diameters.

2. Again, place a drop of urine, preferably of a high specific gravity, on the slide, add a thread and cover-glass, and allow a drop of nitric acid to enter by capillarity. Crystals of the nitrate of urea will form along the thread.

Isolation.—A large quantity of urine, say, 750 c.c., is precipitated by 250 c.c. of baryta mixture, consisting of one volume of a saturated solution of barium nitrate and two of a saturated solution of barium hydroxide. The mixture is filtered, evaporated to a syrup on the water bath, and the syrup extracted with 95 per cent. alcohol. After filtering, the filtrate is boiled with animal charcoal to remove the pigments, and set away to cool and crystallize. Urea forms in crystals which may be recognized by description given above.

THE QUANTITATIVE DETERMINATION OF UREA.

For accurate scientific work the Moerner-Sjöqvist method is preferred. The principle of this method is the precipitation of all of the nitrogenous constituents of urine except the urea and ammonia and the determination of the urea after driving off the ammonia. The process is as follows: pour 5 c.c. of the urine into a flask; add 5 c.c. of a saturated solution of barium chloride which contains 5 per cent. of barium hydrate; to this add 100 c.c. of a mixture of two parts of alcohol (97 per cent.) and one part

of ether, and allow it to stand in the closed flask for twelve hours. The precipitate is then filtered off and washed with alcohol and ether. The alcohol and ether are removed from the filtrate by distillation at about 55° C. (102° F.). When the fluid has become concentrated to about 25 c.c., add a little water and calcined magnesia. Continue the evaporation down to 10 or 15 c.c. or until the vapors are no longer alkaline in reaction. Transfer the liquid to a flask by the aid of a little water, treat a few drops of concentrated sulphuric acid and further concentrate on the water bath. In this concentrated liquid the total nitrogen is determined according to the Kjeldhl method.

(See "Total Nitrogen" above.)

Using the Kjeldahl process, nitrogen in grammes is obtained. To convert grammes of nitrogen into urea, multiply by 2.14.

Folin has modified the above method as follows: treat the fluid (25 c.c.) remaining after carefully evaporating the alcohol-ether mixture at a temperature not above 50° or 55° C. with 2 c.c. of hydrochloric acid of specific gravity 1.124; transfer carefully to a 200 c.c. flask and evaporate the mixture to dryness on a water bath. Add 20 grammes of magnesium chloride and 2 c.c. of concentrated hydrochloric acid to the residue, and after fitting the flask with a return cooler, boil on gauze over a small flame for two hours. Cool; dilute to 750 c.c., or up to 1000, with water. Render alkaline with sodium or potassium hydroxide; distil off the ammonia and collect it in an acid solution of known strength. Boil the distillate to remove carbon dioxide; cool and titrate with an alkali of known strength. Make correction for ammonia originally present in the urine and in the magnesium chloride.

The Folin method for urea is now used by many engaged in research work and is based upon the fact that urea is quantitatively decomposed into ammonia and CO_2 in a solution of magnesium chloride boiling in its own water of crystallization at a temperature of 160° C. (320° F.) within one-half hour.

If the process is carried out in acid solution, the ammonia can subsequently be distilled off after rendering the mixture alkaline, and is then titrated. The corresponding amount of urea

is ascertained by calculation. At the same time, however, the preformed ammonia is obtained, and it is hence necessary to eliminate this source of error by a separate estimation of this form. Three c.c. of urine are placed in an Erlenmeyer flask of 200 c.c. capacity, together with 20 grammes of magnesium chloride and 2 c.c. of concentrated hydrochloric acid. It is necessary to measure the urine very accurately with a pipette graduated in one-twentieths c.c. (The magnesium chloride usually contains a small amount of ammonia, which must be separately determined.) The flask is closed with a perforated stopper through which a specially constructed Folin safety tube passes. The mixture is now boiled until the drops flowing back through the tube produce a hissing sound on coming in contact with the solution. After this point has been reached the boiling is continued more moderately for about forty-five minutes. In order to obviate too great foaming a piece of paraffin about twice the size of a coffee bean is added. The solution while still hot is carefully diluted to about 500 c.c., at first allowing the water to flow drop by drop through the tube; it is then transfered to a 1000 c.c. retort, treated with about 7 or 8 c.c. of a 20 per cent. solution of sodium hydrate, and the ammonia distilled off into a measured amount of a decinormal solution of sulphuric acid. The distillation may be interrupted when about 350 c.c. have passed over (viz., after about sixty minutes). The distillate is boiled for a moment to remove any carbon dioxide which may be present in solution, and on cooling is titrated to determine the excess of acid. Each cubic centimeter of the decinormal ammonia present in the distillate corresponds to 0.003 gramme, viz., to 0.1 per cent. of urea.

From this result the amount of preformed ammonia and of that present in the 20 grammes of magnesium chloride must be deducted. The method is not trustworthy in urine containing sugar, but may be used instead of the Moerner-Sjöqvist method in all other cases.

Since the urine contains preformed ammonia this must be determined by the Folin process, as follows: an aerometer cylinder, 30-40 centimeters high, is taken, and in it is placed 25 c.c.

of urine, and one gramme of dry sodium carbonate, with some crude petroleum to prevent foaming. The neck of the cylinder is stopped with a twice perforated rubber cork, and through each perforation a glass tube is passed, one reaching below the surface of the urine. The shorter tube, 10 centimeters long, is connected with a calcium tube filled with cotton. This latter tube is in turn joined to a glass tube extending to the bottom of a 500 c.c. wide-mouthed flask, which serves the purpose of absorbing the ammonia. This flask contains 20 c.c. of deci-

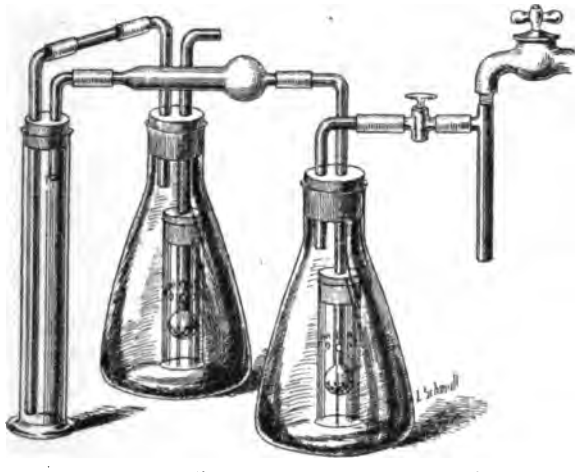


FIG. 9.—Folin Apparatus for Ammonia.

normal sulphuric acid, 200 c.c. of distilled water, and a few drops of alizarin red indicator. It is also provided with a Folin absorption tube which causes the air passing into the cylinder to come into intimate contact with the acid in the absorption flask. The passage of ammonia-free air into the aerometer cylinder is insured by the attachment of a similar absorption apparatus to the other side of the aerometer cylinder.

The apparatus is connected with a filter pump inserted in a water faucet, and with good water pressure the last trace of ammonia can be removed from the aerometer cylinder in about one hour and a half; but it is advisable to ascertain the length of time required for the pump by experiments with a solution of ammonium salt of known strength. (Fig. 9).

The number of c.c. of the decinormal sulphuric acid neutralized by the ammonia of the urine is determined by titration directly with decinormal sodium hydroxide. The number of c.c. of decinormal sodium hydroxide used in the titration subtracted from the number of c.c. of decinormal sulphuric acid originally placed in the flask shows the number of c.c. of decinormal sulphuric acid neutralized by the ammonia in the urine, each c.c. of acid representing 0.0017 gramme of ammonia in 25 c.c. of urine.

The amount of ammonia being found in 25 or 50 c.c., that which would be present in 3 c.c. is calculated and subtracted from the total, determined in the process above. From the remainder the urea is then accurately calculated.

The sample of magnesium chloride from which the 20 grammes is taken must also be tested for ammonia and the amount determined by a "checking" process in which the urine is left out and distilled water substituted for it.

CLINICAL DETERMINATION OF UREA.

☞ The Doremus instrument (Fig. 10), with the solution of sodium hypobromite, is almost universally used.

The Doremus Ureometer.—Either the original Doremus model with the 1 c.c. nipple pipette may be used, or the Hind's instrument (Fig. 11), which has a side arm for the urine. The latter is more expensive, but desirable in other ways. (See Notes below.)

The instrument consists of an upright limb and a lower cup-shaped portion, into the mouth of which the solution to be used is poured. The upright limb is graduated by means of lines, **short** and **long**. Opposite three of the long lines are to be seen the figures 0.01, 0.02, and 0.03, which represent the amounts of urea in 1 c.c. of the urine employed, hence by calculation also 10, 20 or 30 grammes per liter or 1, 2, or 3 per cent. Each long line between the figures represents 0.005, 0.015, and 0.025 respectively, but these figures are not etched on the glass. They also represent 5, 15, and 25 grammes per liter respectively, or 0.5, 1.5, and 2.5 per cent. of urea. Between the long lines are short lines,

each one of which represents 0.001 gramme of urea per c.c. of urine, that is, 1 gramme per liter, or 0.1 per cent.

The solution of hypobromite being poured into the cup-shaped portion of the ureometer until the latter is filled with it, the instrument is then tilted back until the solution runs back and fills completely the upright limb. The instrument is then restored to the upright position and 1 c.c. of urine introduced slowly and cautiously, either by use of the nipple pipette or, if the



FIG. 10.—Doremus Ureometer and Pipette.

Hind's modification is used, from the side arm filled with urine by turning the stop-cock. After the evolution of gas is over and the foam has subsided, wait ten minutes and take the reading.

Taking the Reading on the Doremus Ureometer.—The reading is taken from the level of the depressed liquid, **counting from the nearest long line** and adding to it one gramme per liter or one-tenth of one per cent. for every short line just above the level. Example: the level of the liquid is two short lines beyond

the third long line; each long line represents 5 grammes per liter, or five-tenths of one per cent. of urea; therefore three long lines equal 15 grammes per liter, or 1.5 per cent.; add to this the equivalent of the two short lines, 2 grammes per liter, or 0.2 per cent., and we have 17 grammes per liter or 1.7 per cent. of urea for the correct reading.



FIG. 11.—Hinds' Ureometer with Side-Arm and Glass Stop-Cock.

Calculation of Results.—To express the percentage of urea obtained divide the reading in grammes per liter by ten; thus if the hypobromite is depressed three lines below the long line marked 0.02, then 23 grammes per liter is indicated, which, divided by 10, equals 2.3 per cent. of urea in that sample. To find the amount of urea in 24 hours in grammes multiply the volume of urine in cubic centimeters by the amount of urea in grammes per liter and divide the product by 1,000. Thus, if the volume of urine is 1200 c.c., and the urea reading by the Doremus

process is 14 grammes per liter (4 lines on the instrument below the long line marked 0.01), then 1200 times 14 divided by 1,000 equals 16.80 grammes of urea in 24 hours. To reduce to grains multiply by 15.432, equals in this case 259.26 grains.

Examples for Practice.—(1) In the 24 hours' urine, which is 1920 c.c. in volume, calculate (a) the percentage of urea when the reading indicates 17 grammes per liter and (b) the total urea. Answer: 1.7 per cent. and 29.94 grammes.

(2) In a sample of urine measuring 3570 c.c. in 24 hours calculate (a) the percentage of urea when the hypobromite solution is depressed to the long line marked 0.01, and (b) the total urea. Answer: 1.00 per cent. and 35.70 grammes total.

(3) In a given sample of urine only 2 grammes of urea per liter is found, the amount of the 24 hours' urine being 6,000 c.c. Calculate the percentage and the total urea. Answer: 0.2 per cent. and 12 grammes total.

(4) Convert the total urea in the above examples to grains. Answer: 462,551, 185+.

PRINCIPLE OF THE HYPOBROMITE METHOD.

Urea is decomposed into nitrogen and carbon dioxide, according to the formula:

$$\underset{\text{urea}}{CH_4N_2O} + \underset{\substack{\text{sodium hypo-}\\\text{bromite}}}{3NaBrO} = \underset{\text{nitrogen}}{N_2} + \underset{\text{carbon dioxide}}{CO_2} + 2H_2O + \underset{\substack{\text{sodium}\\\text{bromide}}}{3NaBr}.$$

(This equation is more readily remembered if the 3 before the NaBrO is suggested by the three elements Na, Br, and O). The carbon dioxide formed being soluble in alkalies does not interfere with the process. The ureometer is standardized on the assumption that one gramme of nitrogen is equivalent to 2.14 of urea, and one gramme of urea to 0.46 of nitrogen gas, the latter occupying a volume at the standard temperature of about 371.5 c.c., that is, one c.c. of nitrogen at 0° C. (32° F.) would indicate the presence of 1 divided by 371.5 or 0.0027 grammes of urea.

In order to convert grammes of urea per liter to grains per fluidounce, divide by 2.125. Assume 29.57 c.c. equal to one fluidounce and 473 c.c. to one pint.

Solutions Used.—Since bromine is indispensable in the process the question of handling it is of importance.

I. Rice's Solution is prepared by the dealers and when sold consists of two solutions, No. I being sodium hydroxide 100 grammes in 250 c.c. of water, and No. II. a solution of 1 part bromine and 1 part potassium bromide in 8 of water. When it is desired to make the hypobromite solution, thoroughly mix 5 c.c. of each of the above and add to the mixture 10 c.c. of water, mixing well after addition of the water. Pour into the Doremus ureometer enough of this mixture to fill it. The use of Rice's solutions obviates all necessity on part of the physician of handling the bromine used.

II. Another Method.—A less expensive method is to buy the bromine in a one-pound container bottled by **Larkin & Sheffer, of St. Louis.*** The glass stopper can always be removed without danger to the operator. Next the soda solution required can be made **without handling the caustic alkali by dissolving in 1135 c.c. of water the contents of a one pound bottle, 453.415 grammes,** since the strength required is 100 grammes in 250 c.c. of water. It is well to use two thick glass graduates, each one containing half of the water, and to pour the caustic soda sticks half into one and half into the other. After a time stir with a glass rod in order to prevent the soda from sticking to the glass. Let cool, which requires several hours at ordinary room temperature, then pour into a large bottle provided either with a rubber cork or glass stopper which has been dipped in melted paraffine. Keep the soda solution in a tightly stoppered bottle.

When it is desired to make the hypobromite solution, measure off 10 c.c. of the soda solution, add to it 1 c.c. of bromine removed from the bromine container by means of a 1 c.c. nipple pipette, stir well with a glass rod and add 10 c.c. of water.

If a number of determinations are to be made use 100 c.c. of the soda solution and add 10 c.c. of bromine, **1 c.c. at a time, with stirring** and after addition of each c.c. rinse out the nipple-pipette with water to prevent slipping of the bromine.

*Since writing the above the author has been unable to obtain the bromine bottled by this firm.

It is not necessary to fill the ureometer anew with hypobromite after every determination of urea. The same content may be used for 2 or 3 determinations except, possibly, in the case of urines of high specific gravity. After each determination tip the instrument back and cause the solution to fill the upright limb again.

No more hypobromite should, however, be made up than is required for use in a few days' time, since it loses strength, especially in warm weather. In removing bromine from the container by means of the nipple pipette it is not necessary to obtain exactly 1 c.c. of bromine, but care should be taken that not less than 1 c.c. be obtained each time. The bromine bottle should be close to the soda solution so that the former can be quickly transferred to the latter. Slip the finger of the right hand down to the glass portion of the pipette when removing the bromine, so as not to press accidentally upon the rubber nipple. If any bromine spills out pour some of the soda solution upon it immediately, to prevent inhalation of the fumes.

Technique of the Determination of Urea.—After the Doremus instrument has been filled with hypobromite solution exactly 1 c.c. of urine is introduced into the apparatus. When the 1 c.c. pipette is used for this purpose the best way to obtain *exactly 1 c.c.* is to pour the urine into a beaker which is held in the left hand, while the operator holds the pipette in his right hand. The exact amount can be obtained only by trials, but during these the open end of the pipette must be kept **below the surface** of the urine to exclude air bubbles from the pipette, the only way to secure this object. When finally 1 c.c. is exactly obtained **slip the fingers off from the rubber** and remove the pipette, holding it by the upper part of the glass. When introducing the pipette containing the urine into the Doremus instrument be careful not to press upon the rubber nipple until the curved end of the pipette is well up into the limb, and then only gently. The author prefers the pipettes with the short curve and wide mouth. Air should not be squeezed out of the pipette at the close of the operation.

If the Hinds' modification of the Doremus instrument is used

the following precautions should be observed: excess of urine in the side-arm above the zero point should be removed by filter paper immersed in it and withdrawn. The glass stop-cock must be kept well oiled and should be removed whenever the instrument is washed out. In case for any reason the stop-cock sticks fast heat gently under the hot water faucet. Air bubbles forming near the stop-cock should be removed by gentle shaking or avoided by adding urine a little at a time with shaking.

☞ **Precautions Necessary.**—In all cases where the hypobromite solution is used the urine should be introduced **in small portions at a time** to avoid loss from too violent effervescence. Urines of specific gravity 1025 or upward should be diluted with an equal volume of water and the results multiplied by two. Urines containing sugar should be fermented before the urea is determined, as sugar interferes with the true reading; in some cases observed by the author the error has amounted to 2 grammes per liter, and much higher in cases quoted by other observers.

Urines containing acetone and diacetic acid should be thoroughly boiled before the determination. Urine containing more than one per cent. by bulk of albumin should be boiled after acidulation, with acetic acid if necessary, cooled, and filtered clear before the determination. The same is necessary in the case of urines containing blood. The filtered urine should be made up to the original volume with water.

The process can be "checked" to a certain extent by use of solutions of chemically pure urea of known strength. But the urea used must be freshly made, of white color in the gross, and of no odor of ammonia.

It is almost always safe, if exactly 1 c.c. of urine is used, to record a reading a little larger than actually obtained, *i. e.*, if there is a barely perceptible space between the level of the liquid and the line of the instrument below it, to make the reading correspond with that line.

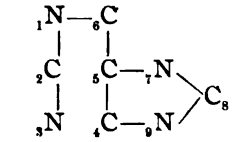
CHAPTER VII.

NITROGENOUS NORMAL CONSTITUENTS OF URINE:—PURINES, AMMONIA AND OTHERS.

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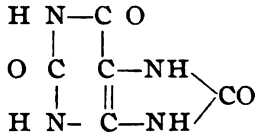
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THE PURINE NUCLEUS.



The bodies containing this nucleus are uric acid and the purine bases, xanthine, hypoxanthine, etc. Of these the only one having even feebly acid properties is uric acid.

URIC ACID.



Chemistry.—Uric acid, $C_5H_4N_4O_3$, has been termed 2, 6, 8-trioxypurine, *i. e.*, purine with oxygen bound to the carbon atoms in the 2d, 6th and 8th places of the purine nucleus. Uric acid

contains 33 per cent. of nitrogen and is a dibasic acid of feebly acid properties. When pure it is a crystalline solid, difficultly soluble in water (1 in 16000, cold; 1 in 1900, boiling), soluble in warm glycerine and alkalies; insoluble in weak acids, in alcohol and in ether. It dissolves in sulphuric acid without decomposition, and in nitric acid with decomposition. In the pure state occurs as a white, odorless, tasteless powder, consisting of very small rhombic prisms or plates. In the urinary sediment it is colored pinkish by urinary pigments. It reduces the alkaline cupric solutions when heated with them.

In the urine free uric acid occurs in solution in but minute quantity, but at times appears abundantly in the sediment, *i. e.*, when freed from combination with the bases by changes in the reaction of the urine. Normally uric acid in solution in the urine occurs in relative abundance in combination-forming **urates.**

THE URATES.

The following table shows the combination of uric acid in the urine, the letter U representing the unchangeable acid radical $C_5H_2N_4O_3$.

Name	Formula	Solubility in cold water
Acid Ammonium	NH_4HU	1 in 1600
Acid Sodium	$NaHU$	1 in 1200
Acid Potassium	KHU	1 in 800
Acid Calcium	CaH_2U_2	1 in 600
Neutral Sodium	Na_2U	1 in 77
Neutral Potassium	K_2U	1 in 44
Neutral Calcium	CaU	1 in 1500

Acid Urates are those in which only one atom of hydrogen has been replaced by the metal, and are less soluble in water than the *neutral urates,* in which two atoms of hydrogen have been replaced by the metal in combination.

These urates, though normally in solution in the urine, may be deposited from the concentrated urines on cooling, forming a

dirty white, clay-colored or brick-dust sediment. From this the urine will have a muddy appearance, described by patients as "thick-looking" but readily cleared by warming the specimen.

In hyperacid urines, uric acid itself appears in the sediment in the form of "red sand" or "red pepper grains," not soluble on warming the urine. This is due to the feeble acid properties of uric acid, other acids readily displacing it from combination. The sediment is also common in urines poor in pigment or low in content of mineral salts.

Acid Urates and Neutral Urates.—Uric acid is soluble in sodium phosphate and is held in solution in the blood by alkaline phosphates. When uric acid is present in the blood in quantity relatively large, it reacts with the disodium phosphate to form acid sodium urate, and when in smaller amount, to form the neutral urate.

Calculi.—Both uric acid and urates occur in urinary calculi. (See Murexide test).

Physiology.—Nucleic acid compounds as a result of destructive cell metabolism yield purine bodies; purine bodies by enzyme action yield uric acid **(endogenous uric acid).**

From the purines in the food also uric acid is formed **(exogenous uric acid).**

It is possible that uric acid is also formed synthetically in man as it has been proved to be in birds. Uric acid formation can hardly be limited to any one tissue or organ. The formation of endogenous uric acid is especially abundant in muscle tissue. The quantity of uric acid which appears in the urine is resultant of the following operative factors: the *formation* of it—endogenous and exogenous—as explained above; the *destruction* of it by uricolytic ferments, particularly in muscle, liver, and kidneys, and the *retention* of it, (a) by deposition in the tissues, (b) by limited excretory capacity. (E. E. Smith.)

Folin has recently claimed that following a pronounced decrease in the amount of protein metabolized the absolute quantity of uric acid is decreased, but not in proportion to the total nitrogen decrease; and that the ratio of uric acid nitrogen to total nitrogen is really increased.

The usual range in the *quantity* of uric acid in the twenty-four hours' urine, calculated as free uric acid, is 0.4 to 0.6 gramme, (6 1/4 to 9 1/3 grains),—exceptionally, from 0.2 to 1.25 grammes. In young and healthy men 0.6 gramme is an average. The excretion in infants and children, though smaller in total, is higher in proportion to weight. The normal ratio of urea to uric acid is from 35 to 1 upward, according to whether the diet is rich or poor in purines. The ratio is clinically of significance only where the amount of uric acid per 24 hours is 0.4 gramme (6¼ grains) or more. In infants the ratio of urea to uric acid may fall below 15 to 1.

Pathological Variations in the Quantity.—Uric acid is **increased** by hearty eating, especially by a protein diet rich in nucleins, meat broths, glandular organs (thymus, pancreas, brains), young flesh; fatty foods, alcoholic drinks; caffein-containing drinks; tea, coffee, cocoa; by diet as above in connection with sedentary life (increased metabolism with limited oxidation); certain drugs; salicylic acid and salicylates, disodium phosphate, colchicum, euonymin, corrosive sublimate; severe muscular exercise with undue fatigue; decrease in the amount of other purines.

Uric acid is **decreased** in the 24 hours' urine by the following: light eating and a purine-free diet, milk, eggs, cheese, rice, wheat bread, sugar, butter, fruits containing quinic acid, as cherries, artificial nitrogenous foods free from nucleins; agents which increase the alkalinity of the blood; alkalies, mineral waters, salines, etc.; certain drugs: urotropin, the iodides, citric acid, quinine in large doses, coal-tar antipyretics, sodium chloride; temporarily by mineral acids and lithium salts; increase in other purine bodies.

Pathological Variations in the Quantity.—Uric acid is **increased** in the 24 hours' urine by the following conditions:

Fevers (leukocyte destruction), **leukemia, cirrhosis of the liver,** the resolution stage of pneumonia; **splenic diseases;** pernicious anemia, malaria, scurvy, rachitis, abdominal tumors, chorea, some cases of diabetes mellitus; dyspneic disorders and diseases interfering with the circulation; hydrothorax, pneumothorax, pressure upward of tumors, marked ascites, chronic heart

diseases, uricolytic failure to destroy exogenous uric acid (possibly also some endogenous); **gastrointestinal toxemias,** neuroses, (neurasthenia, epilepsy, certain insanities), so-called chronic rheumatism, arthritis.

Uric acid is **decreased** by the following conditions: **lead poisoning, advanced renal diseases, during paroxysms of gout**; mild anemias, chlorosis, progressive muscular atrophy, chronic arthritis, some cases of diabetes mellitus, some fevers.

Clinical Notes on Increase of Uric Acid.—Endogenous uric acid is greatly increased in **leukemia** and **cirrhosis of the liver,** 2 grammes in 24 hours being not uncommon and as high as 8 grammes reported. In **pneumonia** the increase is during resolution.

Uric acid is increased in **fevers,** but is somewhat variable in amount, depending mostly upon the degree of leukocyte destruction; but in some cases there is more or less increase of uric acid without leukocytosis, as if coming from the nuclei of the fixed tissues.

In **gout** the amount of uric acid is sometimes high, but more often the quantity is diminished, especially **during** the paroxysm. During the quiescent interval between the attacks the amount is subnormal, sinking again afterwards.

According to Von Hoesslin and Kato injection of sodium nucleinate with the patient on a purine-free diet shows whether arthritic diseases are rheumatic or gouty in nature. If rheumatic there is no anomaly of uric acid metabolism.

☞ An unaccountably persistent increase in uric acid in the urine of women should lead to careful investigation for an **abdominal tumor.** The writer knows of two such patients treated for "lithemia" who really were suffering from ovarian tumors.

In view of the difficulty of obtaining the indican reaction (see "Indican") an increase in uric acid in connection with digestive derangement should suggest careful and repeated search for indican by more than one test or method.

In a case of **suspected arthritis** a diminution in the amount of uric acid during the quiescent intervals followed by a rise during acute symptoms and a fall again on the subsidence of the latter, is strongly suggestive of arthritis as the cause.

TESTS FOR URIC ACID.

The characteristic test for uric acid is the murexide test, which also serves to identify urates.

In order to obtain uric acid from the urine proceed as follows: measure off 200 c.c. (7 fl. oz.) into a porcelain evaporating dish, add 10 c.c. (3 fluidrachms) of chemically pure hydrochloric acid, stir with a glass rod, and set aside in a cool, dark place for 24 to 48 hours. At the end of that time crystals will be observed adhering to the dish. Decant the urine; rub off the crystals, by means of a glass rod having a bit of rubber tubing on the end; and pour the whole into a tapering glass vessel of any kind, using wash-bottle if necessary. Let settle; decant supernatant fluid; add more water; let settle again, and the crystals remaining at the bottom will now be in condition for the murexide test, as follows:

Treat a few crystals with a few drops of nitric acid, which dissolves the uric acid with a strong development of gas (nitrogen and carbon dioxide), and after a thorough drying over the water bath, let cool, and a beautiful red residue (urea and alloxan) is obtained, which turns **purple-red** (murexide or purpurate of ammonia) on addition of a little caustic soda solution; or after cooling, on addition of a little caustic soda solution, a **bluish-violet**, the latter disappearing quickly on warming (differentiation from guanin, etc.).

Note.—The test as above described is not uniformly successful unless the author's technique is employed, as follows: add one cubic centimeter of strong nitric acid to ten c.c. of water; mix thoroughly; pour into a test-tube; add uric acid crystals to the amount, say, of 20 or 30 milligrams (about half a grain); boil thoroughly over a spirit lamp until the uric acid is all dissolved and effervescence ceases. Evaporate to dryness over the water bath; let cool; touch with a rod that has been dipped in ammonia, and a brilliant purple-red appears, changed to a bluish-violet on addition of caustic soda solution. If the uric acid solution in dilute nitric acid is evaporated on a flat porcelain surface one may trace his initials on the residue, using a glass rod that has been dipped in ammonia. The letters stand out in brilliant

purple-red, as compared with the yellowish residue which has not been moistened with the ammonia.

The murexide test is useful in identifying uric acid and urates in urinary calculi.

Schiff's Test.—Dissolve a little uric acid in as small a quantity of sodium carbonate solution as possible; a piece of filtering paper being moistened with some solution of silver nitrate, a drop of the uric acid solution in the sodium carbonate, carried on a glass rod, is made to touch the paper, when a greyish stain of metallic silver appears. The stain is black, if the uric acid is in amount 0.001 per cent. or more.

Moreigne's Reaction.—Moreigne's solution is made by adding 20 grammes of sodium tungstate and 10 grammes of phosphoric acid (Sp. gr. 1.13) to 10 c.c. of water, with boiling for 20 minutes, and addition of water until the original volume of the liquid is restored followed by acidulation with hydrochloric acid. The test consists in adding equal volumes of the solution to a solution of uric acid, with further addition of a few drops of strong potassium hydroxide solution. A blue color indicates presence of uric acid.

Reducing Action.—A strong solution of uric acid in potassium hydroxide, if added to a boiling solution of cupric hydroxide, as Fehling's or Haines', a few drops at a time with boiling after each addition, will reduce the solution with precipitate of cuprous oxide (see Sugar tests). The bismuth tests are not thus affected.

THE QUANTITATIVE DETERMINATION OF URIC ACID.

1. The simplest method is the following: **Cook's centrifugal method**: place 10 c.c. of urine in a Purdy graduated centrifugal tube (Fig. 12), add to this from 0.5 to 1 gramme of crystallized sodium carbonate and 1 or 2 c.c. of strong ammonia water. Shake until the sodium carbonate is dissolved. The earthy phosphates are thus precipitated and may be sedimented by the phosphates Decant all the supernatant urine, (leaving the phosphatic sediment), into another Purdy tube; add to it 2 c.c. of ammonia water and 2 c.c. of ammoniacal silver nitrate made by dissolving

5 grammes of silver nitrate in 100 c.c. of distilled water and adding ammonia water until clear. Urate of silver is now precipitated and must be sedimented in the centrifuge. Pour off the supernatant urine, fill up the tube with at least 5 c.c. of ammonia water, and mix thoroughly. Chlorides are dissolved and pure silver urate obtained. Centrifugalize until the urate precipitate no longer contracts in volume. Each small mark ($\frac{1}{10}$ c.c.) on the Purdy tube represents 0.001176 gramme uric acid in 10 c.c. of urine.



FIG. 12.—Purdy Centrifugal Tube for Percentages.

2. **Ruhemann's Ureometer.**—This instrument has been subjected to considerable criticism and will not be described here. It can be obtained, with directions for use, from dealers in scientific apparatus, as E. H. Sargent & Co.

3. **Heintz's Method.**—The principle is the decomposition of urates by a mineral acid, with liberation of uric acid, which crystallizes out and may be collected and weighed. To 200 c.c. of urine of the whole 24 hours add 10 c.c. of strong hydrochloric

acid; let stand over night or longer; collect the crystals in a filter of known weight; wash; dry not less than an hour at a temperature of 100° C. (212° F.); weigh, and to the difference in weight thus found add 12 milligrammes for correction, (loss in washing).

Remove albumin and excess of mucus beforehand by boiling the urine acidulated with acetic acid, cooling and filtering.

Urines which are cloudy from urates should be warmed to 50° C. (140° F.) before adding the hydrochloric acid.

Urine containing a deposit of uric acid crystals must be filtered on a weighed filter, and the uric acid,—found by drying and weighing,—added to the total uric acid in solution in 24 hours calculated as above from 200 c.c. of this urine.

In urines containing much coloring matter more exact results will be obtained if the crystals on the filter, after being washed with water, are again washed with alcohol.

When the urine is ammoniacal the entire sediment of the 24 hours' quantity obtained after decantation must be dissolved in sufficient hydrochloric acid, then diluted with water in proportion of 20 to 1 of the acid used, and the crystals allowed to form as above. They are collected, dried and weighed as above, but the quantity of uric acid obtained is to be **added** to the amount in 24 hours, reckoned from 200 c.c. of this urine by the uric acid obtained from ammonium urate deposited in the sediment.

Calculation of Results.—Suppose the weight of the dried filter before filtering is 750 milligrammes. After filtering and drying again suppose it to be 820 milligrammes. 820 minus 750 equals 70 milligrammes plus 12 mg. for correction, equals 82 milligrammes of uric acid in 200 c.c. of urine; and 82 times 5, which is 410, equals the amount per liter in milligrammes, or 0.41 grammes per liter. If the total amount of urine in 24 hours is 850 c.c., then 850 times 0.41 divided by 1000 equals 0.34 gramme of uric acid in 24 hours.

The method of Heintz is tedious, hence the value of the Folin method.

☞ **4. Folin's Method.**—The principle of this method is to rid the urine of interfering substances by precipitation and rapid

filtering, after which the uric acid in the filtrate is obtained by precipitation as ammonium urate, which is collected on a filter, washed, dissolved in sulphuric acid, and titrated hot with $\frac{N}{10}$ potassium permanganate solution.

Make up a saturated solution of ammonium sulphate and uranium acetate in water acidulated with acetic acid as follows: dissolve 500 grammes of ammonium sulphate and 5 grammes of uranium acetate in 650 c.c. of distilled water, to which 6 c.c. of glacial acetic have been added. Shake well and let stand in a warm place, but do not heat. After repeated shaking and standing for some time in a warm place the solution becomes clear or deposits but slight sediment. Make up to a liter with distilled water. Measure out 150 c.c. of the 24 hours' urine and add to it 37.5 c.c. of the Folin liquid made as above. Mix well and let stand five minutes only. Filter quickly. Measure off 125 c.c. of filtrate; add 5 c.c. of strongest ammonia water to it in a clean flask or bottle, cork tightly and let stand over night. In the morning pour the whole liquid upon a large filter in a funnel; rinse the bottle or flask well with a solution of 10 per cent. ammonium sulphate; add the rinsings to the liquid on the filter, and wait until it has all run through. Now wash twice with the 10 per cent. ammonium solution in order to remove chlorides. Finally spread the filter paper on the flat of the hand and wash off the urate into a dish by means of a wash bottle and distilled water, warmed, if necessary. The dish should hold about 200 c.c.; when half full the washing should cease. Now add to the 100 c.c. of liquid in the dish 15 c.c. of strong sulphuric acid. The urate is thus dissolved and the solution immediately titrated with $\frac{N}{10}$ permanganate solution until a slight pink lasting ten seconds or more extends throughout the fluid after the addition of two drops of the solution with stirring. Read off the number of c.c. of permanganate used; multiply this number by 3.75, (since each c.c. of permanganate corresponds to 3.75 milligrammes of uric acid); add 3 milligrammes for correction, and the result is milligrammes of uric acid in 100 c.c. of urine, since the 125 c.c. of liquid obtained as above contains only 100 c.c. of urine (80 per cent.). Multiply by 10 to obtain grammes

of uric acid per liter, and by the number of c.c. of urine in 24 hours divided by 1000 to obtain grammes per 24 hours.

Laboratory Note.—By using 300 c.c. of urine in the first place, instead of 150, and adding to it 75 c.c. of the Folin liquid, two determinations may be made, the one checking the other.

☞ The author has prepared a table which shows at a glance the amount of uric acid in grammes per liter corresponding to the usual range of c.c. of permanganate used.

NO. OF C. C. OF PERMANGANATE USED.	GRAMMES OF URIC ACID PER LITER INDICATED.	NO. OF C. C. OF PERMANGANATE USED.	GRAMMES OF URIC ACID PER LITER INDICATED.
1.	0.0675	13.5	0.540
1.5	0.0863	14.	0.555
2.	0.105	14.5	0.573
2.5	0.124	15.	0.590
3.	0.143	15.5	0.612
3.5	0.161	16.	0.630
4.	0.180	16.5	0.650
4.5	0.200	17.	0.668
5.	0.218	17.5	0.686
5.5	0.236	18.	0.705
6.	0.255	18.5	0.724
6.5	0.274	19.	0.743
7.	0.292	19.5	0.761
7.5	0.311	20.	0.780
8.	0.330	20.5	0.800
8.5	0.349	21.	0.818
9.	0.368	21.5	0.836
9.5	0.390	22.	0.855
10.	0.405	22.5	0.874
10.5	0.424	23.	0.893
11.	0.440	23.5	0.910
11.5	0.460	24.	0.930
12.	0.480	24.5	0.948
12.5	0.500	25.	0.968
13.	0.518		

Calculation of Results.—Suppose the number of c.c. of permanganate used to be 20.5, then the amount of uric acid in 100 c.c. of urine is 3.75 times 20.5, equals 76.88. Adding 3 milligrammes,—correction for loss in washing,—equals 79.88, or, roughly, 80 milligrammes. Multiply 80 to 10 = 800 to obtain milligrammes per liter, and divide by 1000 to obtain grammes per liter; equals 0.8. The whole is shown by the table at a glance, as above. Suppose the amount of urine in 24 hours to be 1500 c.c., then 1500 times 0.8, divided by 1,000, equals 1.2 grammes of uric acid in the 24 hours' urine, or 1.2 times 15.432 = 18.52 grains.

The usual normal range of uric acid being from 0.4 to 0.6 gr. the amount obtained above is fully double the normal.

Laboratory Notes.—In order to prepare the **twentieth normal solution of potassium permanganate** it is necessary to have ready the decinormal oxalic solution already described. A normal oxidizing solution, as e. g. permanganate, is one which will liberate 8 grammes of oxygen per liter, since it takes 2 of hydrogen to combine with one of oxygen. A normal solution of permanganate, $K_2Mn_2O_8$ therefore contains $\frac{1}{10}$, its molecular weight ($\frac{1}{2} \div 5$) or 31.534 gr. per liter. A decinormal solution should contain, then, 3.153 grammes per liter, and the twentieth normal, 1.576. Weigh out pure recrystallized permanganate in amount about 1.580 grammes. Dissolve in 1000 c.c. of water; boil; cool, and titrate with decinormal oxalic acid as follows: measure out 10 c.c. of decinormal oxalic acid; dilute to 100 c.c. with distilled water; add 15 c.c. of strong sulphuric acid, and run in the permanganate solution drop by drop until a uniform pink color appears, which lasts about thirty seconds. When the first few drops of permanganate are added the color remains longer than it does later on, owing to the fact that the oxidizing of the uric acid is much helped by an increased percentage of manganese sulphate.

Read off the number of c.c. of permanganate necessary to produce the end-reaction as above, and then dilute the whole supply of permanganate until 20 c.c. of it exactly neutralizes

10 c.c. of the oxalic acid, according to the formula $C = \frac{N.d}{n}$, in which "C" will represent the number of c.c. of water to be used for dilution, "N" the total amount of permanganate on hand, "n" the number of c.c. of permanganate producing the end-reaction during the trial as above, and "d" the difference between 20 and "n," the former being the number of c.c. of permanganate which theoretically should neutralize 10 c.c. of the oxalic acid.

Example: suppose in the trial as above 16 c.c. of permanganate produced the end-reaction and that 984 c.c. of the permanganate were left on hand, then

$$C = \frac{984 \text{ times } 4}{16} = \frac{3936}{16} = 246$$

Dilute the 984 c.c. of permanganate with 246 c.c. of distilled water, in order to obtain a solution 20 c.c. of which shall neutralize 10 c.c. of the decinormal oxalic acid solution. It is always well after dilution to test the strength again as above in order to be sure that no mistake has been made.

The permanganate solution must be kept in an amber bottle and should be made up fresh every few months, or else retitrated with a freshly made decinormal solution of oxalic acid.

Precautions to be Observed.—The Folin method is not trustworthy in the case of ammoniacal urine, since much of the urate exists in the sediment in the form of ammonium urate. In such cases determine the uric acid in solution by the Folin method and the uric acid in the sediment by the Heintz method described above; dissolve the entire sediment of the 24 hours' urine in excess of hydrochloric acid; dilute with water in proportion of 20 to 1 of the acid used and allow the crystals to form: the quantity of uric acid obtained by weighing is added to the total uric acid in solution in 24 hours, determined by the Folin method.

Where there is an abundant deposit of urates in the urine this must be dissolved by warming before precipitation with the Folin test liquid.

When there is an abundant sediment of uric acid crystals decant the supernatant urine; add a little sodium or lithium car-

bonate to the sediment; dissolve by warming, and add the solution thus obtained to the decanted urine.

In all filtrations the filtrates should be clear. Sargent's No. 500 filter paper will usually be found satisfactory for this purpose, especially if two of the papers be folded together.

In the final washing cold water is usually sufficient to remove the urate from the paper, but if the urate adheres, hot water is needed.

☞ To make up a large quantity of the test liquid dissolve 2 kilogrammes (4.4 pounds) of pure ammonium sulphate, 20 grammes of uranium acetate (sodium-free not necessary) in 2600 c.c. of distilled water, to which 24 c.c. of glacial acetic acid have been added. Make up to 4000 c.c. with distilled water.

To make up a large quantity of the 10 per cent. ammonium sulphate solution dissolve 300 grammes in 2700 c.c. of water, or one pound in nine pints of water.

5. The Ludwig Salkowski Method.—This method is the standard one for accurate work, but is not needed clinically, since the Folin method is sufficiently accurate. The principle of the method is the following: **In an ammoniacal solution silver salts precipitate uric acid in the presence of magnesium salts, as a double urate,** *i. e.*, as silver-magnesium urate. If this precipitate is treated with an alkaline sulphide the silver is then precipitated as silver sulphide, while the uric acid goes into solution as alkaline urate. On acidifying the urate solution uric acid crystallizes out and may be weighed, as in Heintz's method. Three solutions are required, as follows: (1) Ammoniacal silver nitrate: dissolve 20 grammes of silver nitrate in water, and add ammonia water until the brown precipitate forming is dissolved in excess of ammonia. Make up to 1000 c.c. and keep in an amber bottle. (2) Magnesia mixture: dissolve 100 grammes of magnesium chloride crystals in distilled water and add a great excess of ammonia water, causing magnesium hydroxide to be precipitated. Add cold saturated solution of ammonium chloride until the precipitate is dissolved, and make up to 1000 c.c. (3) Sodium sulphide solution: dissolve 10 grammes of sodium hydroxide in 1000 c.c. of water. Saturate half of this with hy-

drogen sulphide gas and add it to the other half. The strength of these solutions is such that 10 c.c. of either one will precipitate and later redissolve all the uric acid in 100 c.c. of urine. To determine the amount of uric acid, proceed as follows: mix 10 c.c. of the silver solution with a like amount of the magnesia mixture; add ammonia water until the curdy silver chloride is all dissolved, leaving only a flocculent precipitate of magnesium hydroxide. Slowly pour the liquid thus prepared into 100 c.c. of urine in a beaker.

Let the precipitate which forms settle; filter, and wash several times with weak ammonia water; lastly transfer to a beaker and treat with a boiling mixture of 10 c.c. of the sulphide solution and 10 c.c. of water, which is best done by punching a hole in the filter paper, washing the precipitate through into a beaker, then pouring the hot mixture through the filter, which shows any traces of precipitate left behind by blackening it. The contents of the beaker are now slowly heated to boiling; the precipitate of silver sulphide filtered off and repeatedly washed with hot water; washings and filtrate acidulated with dilute hydrochloric acid (5 c.c. of acid, sp. gr. 1.19, diluted four times, sufficient) and evaporated down to about 20 c.c. On cooling the uric acid crystallizes out; it should be pure white. The solution should be allowed to stand at least six hours before the crystals are filtered off. The crystals are collected on a weighed filter, washed with very little water, then with alcohol, then with ether; dried at 110° C., and weighed. For every 10 c.c. of the mother liquor and water washings, 0.0005 gramme of uric acid should be added, for this small amount of uric acid is soluble in 10 c.c. of acidulated water.

The amount of uric acid obtained represents the quantity in 100 c.c. of urine; hence to obtain the entire quantity in grammes in 24 hours, multiply grammes in 100 c.c. of urine by the number of c.c. of urine in 24 hours, divided by 1,000.

Sugar must be removed by fermenting before applying this method.

Albumin is to be removed also by salting the urine and acidulating with acetic acid. Add 15 c.c. of a saturated common salt

solution to each 100 c.c. of urine; make acid with acetic acid; boil; cool; filter; wash with hot water, and make up the filtrate and washings to 100 c.c. with water.

Uric acid crystals and urate deposits are to be dissolved by warming the urine and adding sodium carbonate.

6. Aufrecht's Method.—An amount of 25 c.c. of the urine is condensed to about $^1/_5$ its original volume in an evaporating dish on a water bath. The residue is placed in a special centrifuge tube to which is added some distilled water. (These tubes are manufactured by a Berlin firm). A saturated solution (35 to 100) of ammonium chloride is then added. After the cooled contents of the tube have been well mixed they are centrifuged for from 3 to 5 minutes. The clear supernatant fluid is then decanted, and the brownish-yellow sediment to which has been added 5 c.c. of ammonium sulphate (saturated solution?) is again centrifuged. Following this the clear natant fluid is again decanted and some more of the ammonium sulphate solution is added; renewed centrifuging for a few minutes. In order to obtain a deposit free from chlorine it is best to subject it once more to the same process. Following this, the sediment consisting of ammonium urate is dissolved in about 10 c.c. of a warmed sodium carbonate solution; the solution is then placed in a beaker, acidulated with 5 c.c. concentrated sulphuric acid, heated to boiling and quickly titrated with 1/100 potassium permanganate solution until the appearance of a red coloration. Every cubic centimeter of the employed 1/100 normal permanganate solution corresponds to 0.74 milligramme uric acid. The entire manipulation is finished in not more than one hour. If the urine contains coagulable albumin it is necessary to acidulate it in advance with enough acetic acid so that the albumin will be separated when the urine is boiled. (Archives of Diagnosis.)

THE PURINE BASES.

These are also known as the alloxuric, xanthine or nuclein bases.

Chemistry.—The members of this group are xanthine, hypoxanthine, heteroxanthine, paraxanthine. In addition there are

also I-methylxanthine, guanine, epiguanine, episarkine, adenine, carnine, and an unknown base discovered by Krueger. The main bulk of the purine content is made up by paraxanthine, heteroxanthine, and I-methylxanthine. They all contain the purine nucleus and are closely related to uric acid.

Physiology.—The purine bases, like uric acid, are all formed from the nucleins, occur in the urine in small amounts, varying from 15 to 60 milligrammes, or at most 112, being derived chiefly from the caffeine, theobromine, and theophylline of the food, and from articles rich in nucleins.

Pathology.—They are increased in leukemia, enteritis, tuberculosis, and as a result of x-ray treatment. They are decreased in autointoxication of intestinal origin.

Detection.—Camerer's purinometer is the result of an effort to popularize the study of the purines. It can be had, with directions for use, from importers in large cities. With the patient on a purine-free diet it gives an approximate determination of the amount of endogenous purines. Tables are given for the calculation of the purine-nitrogen from the depth of the precipitate, 4 c.c. indicating 0.0078 per cent. of nitrogen, 10 c.c. 0.0185, etc. Multiplication by the number of c.c. of urine in 24 hours gives the total purine-nitrogen per 24 hours.

Quantitative Determination.—The method of Krueger and Schmidt is based on the principle of precipitating both the uric acid and purine bases in combination with copper oxide and the subsequent decomposition of this combination by sodium sulphide. The uric acid is precipitated by hydrochloric acid and the bases separated from the filtrate as copper compounds. The nitrogen of each is then determined by the usual Kjeldahl method.

The method is carried out as follows: the entire urine for 24 hours is measured, and diluted, if necessary, so that the volume is from 1600-2000 c.c. Of this 500 c.c. are taken, acidulated with acetic acid, boiled, and, if albumin is present, filtered. Then 400 c.c. of the filtrate—or of the original diluted urine in case albumin be absent,—is taken, and to it, in a flask holding one liter, are added 24 grammes of sodium acetate and 40 c.c. of a 33 per cent. solution of sodium bisulphite (commercial will do),

and the mixture heated to boiling. According to the strength of the solution in purine bases from 40 c.c. to 80 c.c. of a ten per cent. cupric sulphate solution are added and the mixture boiled for three minutes. This precipitates the uric acid and the purines in combination with copper oxide. The precipitate is filtered off, washed with hot water until the washings are colorless, returned to the original flask by washing with hot water through a hole in the filter, the volume made up to 200 c.c., and the mixture heated to boiling. Next are added 30 c.c. of a solution of sodium sulphide, made by saturating a 1 per cent. solution of sodium hydroxide with H_2S and adding an equal volume of the solution of hydroxide (one per cent.). The solution must be strong enough to give a dark brown with a drop of lead acetate solution.

After decomposition is complete the mixture should be acidified with acetic acid and heated to boiling until the separating sulphur collects in a mass. Filter the hot fluid by means of a filter pump; wash with hot water; add 10 c.c. of ten per cent. hydrochloric acid, and evaporate the filtrate in a porcelain dish until the total volume has been reduced to about ten cubic centimeters. Let this residue stand about two hours for the separation of the uric acid, leaving the purine bases in solution. Filter off the precipitate of uric acid, using a small filter paper, and wash the uric acid with water acidulated with sulphuric acid until the total volume of the original filtrate and wash water aggregates 75 c.c. Determine the nitrogen content of the precipitate by means of the Kjeldahl method, and calculate the uric acid equivalent by multiplying the nitrogen value by 3, adding 3.5 milligrammes to the product as correction for the uric acid remaining in solution in the 75 c.c. The purine bases are in the 75 c.c. of the filtrate, and must be determined as follows:

Make the filtrate from the uric acid crystals alkaline with sodium hydroxide; add acetic acid until faintly acid, and warm to 70° C. (158° F.). Now add 1 cubic centimeter of a 10 per cent. solution of acetic acid and 10 c.c. of a suspension of manganese dioxide, (made by heating an 0.5 per cent. solution of permanganate with a little alcohol until decolorized), to oxidize

the traces of uric acid which remain in the solution. Stir or shake the mixture for one minute; add 10 c.c. of the sodium bisulphite solution so as to dissolve excess of manganese dioxide and 5 c.c. of a 10 per cent. solution of cupric sulphate, and heat the mixture to boiling for three minutes. Filter off the precipitate; wash with hot water, and determine the nitrogen by the Kjeldahl method. Since the purine bases vary in composition and relative amount, the result must be expressed in terms of nitrogen only.

AMMONIA.

Next to urea, ammonia is the most important nitrogenous substance in urine.

Chemistry.—Normally ammonia is present in the urine as ammonium chloride NH_4Cl, ammonium phosphate $(NH_4)_3PO^4$ or ammonium sulphate $(NH_4)_2SO_4$.

It represents from 3.5 to 7 per cent. of the total nitrogen, the average being usually reckoned as 5.

Physiology.—Normally there are split off from proteins organic compounds containing substituted ammonium radicals, which latter are rapidly converted by the liver into urea, but a small unoxidized portion escapes conversion and appears in the urine as ammonium chloride, phosphate or sulphate. Traces may also be absorbed from the inspired air in the lungs and excreted. Folin has recently claimed that when the food is of such a nature as to yield an alkaline ash a pronounced decrease in protein metabolism is always accompanied by a relative increase of ammonia-nitrogen. **The average quantity** per 24 hours is 0.7 gramme; the range, 0.3 to 1.2 grammes. The coefficient of ammonia-nitrogen averages about 5 per 100; *i. e.*, the ratio of ammonia-nitrogen to total nitrogen is as 5:100.

The clinical ratio of urea (Doremus instrument) to ammonia (process of Malfutti) is normally about 30 to 1, the range being from 20 to 1, upward.

Physiological Variations in Amount.—Owing to the affinity of the ammonium radical for acids, ammonia is increased in the urine by the administration of mineral acids (incapable of fur-

ther oxidation), by diet rich in protein (formation of phosphoric and sulphuric acids from the albumin), and by violent muscular exercise. In other words, **acidulation of the body increases ammonia in the urine.**

Clinical Note.—Falk and Hesky have shown that during pregnancy there is a relative increase in ammonia and in the nitrogen combined with the omino acids and peptides.

Pathological Variations in Quantity.—Ammonia is **increased** by the following conditions: increased and imperfect protein metabolism with relative acidulation of the blood; fevers; inability of the liver to convert ammonium compounds into urea; serious functional and organic diseases of the liver, as hepatic cirrhosis; effort of the body to neutralize acidosis; **diabetes mellitus, with lipacidemia,** acute yellow atrophy of the liver; phosphorus poisoning; dyspneic attacks; periodic insanity; somewhat during pregnancy; **pernicious vomiting of pregnancy.**

☞ **Clinical Notes.**—In **diabetes mellitus** the ammonia excretion is **in some cases** a valuable clinical guide. A large amount of ammonia (above 2 grammes) is an unfavorable sign, especially if there is a rise in the ammonia-nitrogen coefficient.

An increase in ammonia above 2.5 grammes is very serious in diabetics over 50 years of age, as it is also in children.

☞ **Clinically,** a low ratio of urea (Doremus' instrument) to ammonia (Malfutti process) is, therefore, an unfavorable sign. Diabetic patients in whose urine this ratio is **persistently** low, as, e. g., around 10 to 1 do not live long.

A figure of ammonia from 1.5 to 2 grammes is abnormal with a protein metabolism of 100 grammes, but normal for 200 grammes. Hence it is well to calculate the amount of protein in the foods by the table of values given. (See "Total Nitrogen.") The metabolism can be calculated also by multiplying the urea in grammes per 24 hours by 3, or the nitrogen by 6.25.

The highest figure of ammonia reported in diabetes mellitus is said to be 7.5 grammes; an excretion of 5 grammes denotes a serious condition.

Some diabetics, however, have lost the power to split off ammonium radicals, hence it may be unsafe to reason that the case is not serious because of a low ammonia figure.

In the pernicious vomiting of pregnancy Williams, of Baltimore, has found an increase of ammonia of from 20 to 40 per cent. together with a decrease in the amount of urea.

☞ **Determination of the Quantity of Ammonia.**—The only rapid clinical method is that of Malfutti, which depends upon the formation of "urotropin," as follows: determine the acidity of the urine with the decinormal sodium hydroxide solution, using preferably the Folin method, as in Chapter III, and set the flask or dish aside. Next measure out 5 c.c. of formalin in a small beaker; dilute it with 5 c.c. of water; mix well; add one drop of phenolphthalein solution, and run in from a burette the decinormal soda solution until a faint permanent pink is obtained. Next pour this formalin mixture into the urine of which the acidity has been determined, and observe disappearance of color. Lastly titrate this urine-formalin decolorized mixture with the decinormal soda solution until the destroyed pink color reappears. Each cubic centimeter of decinormal soda solution used corresponds to 0.0017 gramme of ammonia in 25 c.c. of urine, supposing 25 c.c. of urine to have been used, as in Folin's process for acidity. Multiplication by 4 gives per cent.

Calculation of Results.—Suppose 8 c.c. of the soda solution to be necessary for the final titration; then 0.0017 times 8 = 0.0136 gramme of ammonia in 25 c.c. of urine, or 0.0136 times 4 = 0.0544 gramme in 100 c.c., which equals 0.0544 per cent., or 0.544 gramme of ammonia per liter (1,000 c.c.). If the amount of urine per 24 hours is 1200 c.c., then 0.544 times 1200 divided by 1000 = 0.65 gramme of ammonia in 24 hours, and 0.55 times 15.432 equals 9.83 grains of ammonia per 24 hours.

Laboratory Notes.—Harrower, of Chicago, has compared this method eight times with the Folin method (see below) and has found it to agree in results.

It is convenient to make the ammonia determination as above described at the same time with the determination of the acidity.

Brown's Modification.—E. W. Brown modifies the formalin method as follows: 60 c.c. of filtered urine are treated with 3 grammes of basic lead acetate, well stirred, allowed to stand a few minutes, and filtered. The filtrate is treated with 2 grammes of

neutral potassium oxalate, again well stirred, and filtered. Ten c.c. of the clear filtrate are diluted to about 50 c.c. with distilled water and a few drops of 1 per cent. phenolphthalein added. The fluid will be slightly alkaline or acid,—more frequently the latter. Fifteen grammes of neutral potassium oxalate are added, thoroughly stirred, and the specimen exactly neutralized with one-tenth normal sodium hydroxide or sulphuric acid; 20 c.c. of the 20 per cent. commercial formalin are made neutral in the usual way by addition of the decinormal sodium hydroxide added to the urine mixture, and the solution is tritrated again with the decinormal soda solution. The calculation is made as above with the factor 0.0017.

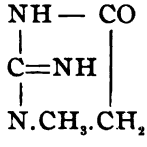
The Folin Method.—This has already been described under Urea.

☞ **Laboratory Note.**—It goes without saying that in order to make the determination of ammonia of clinical value, care should be taken to prevent ammoniacal decomposition by keeping the collected urine in tightly stoppered containers in a cool place during the 24 hours of the collection, and to make the analysis promptly when the urine is received.

CREATININE.

Creatinine occurs in the urine and is the anhydride of creatine, but no one has been able, thus far, to prove that the body is able to convert creatine directly into creatinine.

Chemistry.—The rational formula for creatinine is



the empirical, $C_4H_7N_3O$. In alkaline urine it is said to be replaced by creatine. Creatinine crystallizes in colorless, glistening, monoclinic prisms, soluble in 12 parts cold water; more soluble in warm water and in warm alcohol. An alcoholic solution treated with zinc chloride in acid solution forms creatinine-zinc chloride $(C_4H_7N_3O)_2.ZnCl_2$. Creatinine reduces cupric hy-

droxide, and may interfere with Fehling's or Haines' tests for sugar, but does not affect the bismuth tests nor Benedict's.

Physiology.—According to Shaffer, creatinine in the urine is the result of some normal process of normal metabolism which takes place to a large extent if not entirely in the muscles, and he holds that normally the elimination of creatinine may be regarded as an index of this activity expressed in milligrammes per kilogramme of body weight. His coefficient, therefore, is expressed by a fraction the numerator of which is the creatinine-nitrogen in mg. per 24 hours, and the denominator the number of kilogrammes of body weight. He regards the muscular efficiency of a given person as measured by this coefficient.

The normal range per 24 hours is from 0.6 to 1.3 grammes, or about that of ammonia, the average being 1 gramme.

Creatinine occurs for the most part dissolved in the urine.

Rarely it is a constituent of the sediment.

Creatinine is increased by a meat diet, and probably by muscular exercise.

Clinical Significance.—It is increased in fevers, diabetes, and anemias. The excretion in cachexias is disputed. As much as 2 grammes per 24 hours have been found in diabetes mellitus.

Creatinine is decreased by hunger, in convalescence from acute diseases, in advanced renal diseases, and in wasting diseases (as muscular atrophy). A low excretion of creatinine has been found by Shaffer in a large number of pathological conditions.

Creatine.—According to Ellis creatine is found in the urine in acromegaly. Krause also finds it in the urine of diabetes mellitus.

Clinical Tests for Creatinine.—There are several ready methods for showing the presence of creatinine:

1. **Jaffe's Test.**—In the absence of acetone and sugar picric acid in aqueous solution gives an intense red color in urine made alkaline by sodium hydroxide. The color is changed to yellow by acids.

2. **Weyl's Test.**—To 5 c.c. of urine in a test tube add sodium nitroprusside [the author adds two or three small crystals] and further—after the crystals are dissolved—add a few drops of

weak sodium hydroxide [the author uses the solution of sodium hydroxide made up for the hypobromite-urea test]. The urine turns a ruby red, soon changing to a yellow, but if glacial acetic acid be quickly added the red is replaced by a green color, deepening to a Berlin blue. Acetone must be absent or a deep red will mask the green.

Quantitative Determination of Creatinine.—Since the method of determining creatinine by obtaining the double salt with zinc is none too accurate and somewhat tedious, it will not be described here. Folin has devised a colorimetric method based on Jaffé's reaction with picric acid in alkaline solution: a solution of half-normal potassium dichromate is made containing 24.55 grammes per liter. A chromometer or colorimeter by means of which the depths of color of both the unknown solution and the dichromate can be adjusted to tenths of millimeters is necessary for the method. Solutions required are,—beside the dichromate,—10 per cent. sodium hydroxide, and a saturated solution of picric acid. Ten c.c. of urine are mixed with 15 c.c. of the picric acid solution and 5 c.c. of the sodium hydroxide.

After five minutes the mixture is diluted to 500 c.c. and at once compared with the standard dichromate solution.

Normally 8.1 millimeters of the urine will exactly equal in color 8 m.m. of the dichromate solution, hence if 7.5 m.m. of the urine-picrate solution were equal in color to 8 m.m. of the standard, then 10 c.c. of urine would contain 10 times $\frac{8}{7.5} = 10.8$ milligrammes of creatinine, or 1.08 grammes per liter.

The following precautions are to be observed in the determination:

1. Make first a preliminary colorimetric observation, using half normal potassium bichromate solution in both cylinders of the colorimeter, adjusting first one to the 8 m.m. mark. The average of three or four readings of the other cylinder should also be 8 m.m., and after the first observation no two should differ by more than 0.2 m.m. This preliminary observation takes only two or three minutes, and is exceedingly useful in making the eye sure of the correct point to be ascertained.

2. Exactly 8 m.m. of the half-normal potassium dichromate

solution must be used as the standard for comparison; 16 or 24 m.m., for example, cannot be substituted on the basis of the calculation given above, because the creatinine picrate solution absorbs light at an entirely different rate from the dichromate solution.

3. For the reason given in the preceding paragraph it is necessary to make each determination with a quantity of urine containing not less than 5 nor more than 15 milligrammes of creatinine. Within these limits the determination as described is correct within 0.2 milligramme.

4. Sugar and albumin do not interfere with the determination. Acetone, diacetic acid, and hydrogen sulphide interfere. Where these are present the urine should be measured into a porcelain



FIG. 13.—Duboscq Colorimeter.

evaporating dish and heated on a water bath with 10 c.c. of 10 per cent. hydrochloric acid for about half an hour. When the dish is again cooled, the reagents are added directly into the dish, and finally rinsed into the volumetric flask for about five minutes.

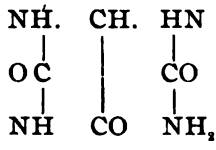
5. The color due to the urine is ordinarily of no appreciable consequence because of the great dilution. Urines containing bile-pigments can, however, be cleared by the addition of egg-albumin and then removing this by coagulation (heat).

☞ The colorimeter of Duboscq is recommended for this process. (Fig. 13.)

Determination of Creatine.—To 20 c.c. of urine in a 50

c.c. flask add 20 c.c. of normal hydrochloric acid. Keep in an autoclave at a temperature of from 117°-120° C. (243°-248° F.) for half an hour. Make up with distilled water to the 50 c.c. mark. Close with a stopper, shake well, neutralize 25 c.c. with normal sodium hydroxide; pour into a 500 c.c. flask, and determine the creatinine as above. Subtract from this creatinine value that obtained before hydrolysis and the difference will represent the creatine in terms of creatinine.

Laboratory Note.—A doubtful reaction for sugar with Haines' or Fehling's test, together with a negative bismuth test, suggests excess of creatinine and demands investigation by the Folin method or use of other tests.



Allantöin, $C_4H_6N_4O_3$, occurs in the urine of new born infants, and in traces in that of adults. It is an oxidation product of uric acid. It reduces the alkaline cupric solutions. It has been found in urine in large amounts after feeding with sweetbreads. Meat diet increases it, as does administration of tannic acid. It has been found considerably increased in diabetes insipidus and in hystero-epilepsy.

Isolation.—The urine is precipitated with baryta water, filtered at once, and the filtrate exactly neutralized with sulphuric acid, and then evaporated until crystals begin to form.

Treat with sufficient alcohol while still warm, to precipitate completely. Decant off the alcoholic solution and completely precipitate with ether. Both precipitates contain allantöin and may be extracted with a little cold water or hot alcohol, the allantöin remaining undissolved. Recrystallize the residue from treatment with hot water and characteristic crystals of allantöin are obtained, large monoclinic prisms with hexagonal bases often arranged in star-like groups, or impure, in warty granular particles.

Quantitative Determination.—Add basic lead acetate to about

100 c.c. or less of urine until no more precipitate forms. Filter and pass H_2S through a measured volume of the filtrate so as to remove excess of lead; filter again; heat until all H_2S is driven off, and add to a measured volume of the filtrate 20-30 c.c. of a 10 per cent. silver nitrate solution or until precipitation is complete. Filter; wash with water; and transfer to a Kjeldahl flask for determination of the purine nitrogen.

Using litmus as an indicator, render another volume of the filtrate feebly alkaline with weak ammonia water (1 per cent.) and add 10 per cent. silver nitrate solution (50-100 c.c.). Filter; wash the precipitate with weak sodium sulphate (1 per cent.) until free from ammonia; transfer to a Kjeldahl apparatus, and determine the nitrogen, which in this case is derived from the allantöin. (Method of Paduschka modified by Underhill and Kleiner).

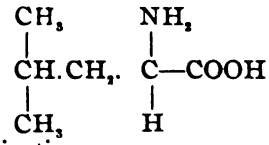
Nucleic acid occurs in very small quantities in the urine but in larger amount in combination with albumin, forming nucleo-albumin. The detection of the nucleic acids depends upon the isolation of phosphoric acid and xanthine.

Hippuric acid is more properly a compound glycocoll. (See Compound Glycocolls.)

MON-AMINO ACIDS.

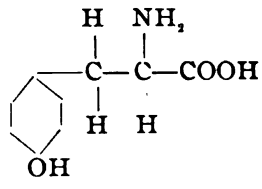
These are leucine, tyrosine and glycocoll, formerly called amido-acids.

Leucine, $C_6H_{13}NO_2$, alpha-amino-isobutyl-acetic acid,



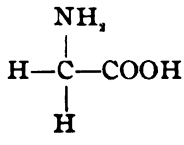
is a caproic acid derivative.

Tyrosine, $C_9H_{11}NO_3$, para-oxyphenyl-alpha-amino-propionic acid,



is one of the first discovered end-products of protein decomposition.

Glycocoll, $C_2H_5NO_2$, amino-acetic acid



is the simplest of the amino-acids.

After the total nitrogen of the urine has been determined and separate determinations (nitrogen partition) made of the quantity of urea, uric acid, ammonia, and creatinine respectively, it is often found that the sum of the last four in nitrogen, subtracted from the total-nitrogen, leaves a remainder **(undetermined nitrogen)**, due in large measure to these mon-amino acids. According to Jaksch, from 1.52 to 3.61 per cent. of the total nitrogen is due to the amino-acids. Leucine and tyrosine are formed from the disintegration of proteins and are due to the activity of certain ferments, especially trypsin. Glycocoll in the past was thought to occur in the urine only in combination with aromatics and only after ingestion of certain drugs, (see Aromatics); but according to Von Noorden it is a normal constituent, contributing in some cases as much as 1 per cent. of the total nitrogen.

Clinical Significance.—The amino-acids (leucine, tyrosine) are increased in all organic hepatic diseases, especially in severe cirrhosis, and in acute yellow atrophy; in phosphorus poisoning, typhoid fever, and diabetes mellitus.

Glycocoll has been found constantly in the urine of gout.

☞ In the toxemias of pregnancy it is claimed that the urea decreases and the undetermined nitrogen (amino-acids) increases, while the ammonia varies. In typhoid fever and in diabetes mellitus as much as 0.5 to 0.6 gramme of the amino-acids (leucine, tyrosine) has been found in 24 hours.

In a variety of intestinal diseases, including tuberculosis, and in severe smallpox, leucine and tyrosine have been found in quantity; also in pernicious anemia and leukemia.

Falk and Hesky have shown that during pregnancy the nitro-

gen of the amino-acids and of the peptides (combination ot two or more amino-acids) is relatively increased in about three-quarters of the cases.

In the urine of eclamptic women shortly after delivery the peptid nitrogen is often still markedly increased and declines very gradually. The increase of amino-acid nitrogen is due to hepatic disturbance ensuing during gestation.

Chemical Tests.—When leucine and tyrosine occur in quantity they are likely to appear as crystals. (See "Sediments.") When their presence is suspected but no crystals are found, the urine may be evaporated on the water-bath to one-tenth its volume; on addition of alcohol the crystals will usually appear.

The microscope is not always sufficient for recognition of these bodies in the urine until certain chemical procedures have been employed, as follows: procure freshly voided urine; remove all albumin by heat and acid; and examine the filtrate, to which first neutral then basic lead acetate is added, until no further precipitate forms. Filter; precipitate the lead with H_2S; filter again, and concentrate the filtrate to a small volume. The urea should then be dissolved out in absolute alcohol, the residue boiled with weak ammoniacal alcohol, and the filtrate evaporated to small volume and allowed to crystallize. The crystals of leucine present themselves as spherules, those of tyrosine as bundles of needles arranged like sheaves of wheat, or, from ammoniacal alcohol, as bunches of prisms. (See "Sediments.")

CHAPTER VIII.

THE INORGANIC NORMAL CONSTITUENTS OF URINE.

Inorganic compounds in urine: chlorides, phosphates, sulphates, carbonates, etc.
Proportion of the bases united with the acids.
Quantitative determination of the urinary ash.
Compounds of hydrochloric acid: the chlorides.
Chemistry of sodium chloride.
Physiology of sodium chloride; source and amount.
Physiological variations in amount.
Pathological variations in amount; effect of diabetes, pneumonia, etc.
Clinical notes on retention of chlorides in fevers, chronic diseases, etc.
Chloruremia in albuminuria, etc.
Ratio of urea to chlorides in gastric diseases.
Therapeutic hint on withdrawal of chlorides from diet in nephritis.
Medico-legal note on cases of malingering.
Clinical test for chlorides with silver nitrate.
The "compact ball" method of rough estimation.
The Purdy centrifugal method of rough estimation.
Quantitative determination by silver nitrate and chromate.
Quantitative determination by Volhard-Arnold and Luetke methods.
Preparation of solutions and technique, Volhard-Arnold and Luetke methods.
The Luetke method and its advantages; author's modification.
Author's Table of Chlorides per number of c.c.'s of thiocyanate used.
Treatment of urea containing albumin, by author.
The Dehn-Clark determination given by Hawk.

The inorganic constituents of normal urine are compounds of the acids, hydrochloric, phosphoric, sulphuric, carbonic, and traces of others (nitrous, nitric, hydrofluoric and silicic), with the bases potassium, sodium, ammonium, calcium and magnesium. (Iron also occurs, but is in organic combination.) "These mineral constituents of urine form an integral part of protoplasm, and their presence is essential to the carrying out of life processes." The proper proportion in the blood and tissues is maintained by the regular excretion of them in the urine.

The acids unite in varying proportions with the bases, forming monobasic, dibasic and tribasic salts and also double salts. On an average mixed diet von Noorden claims the following figures of excretion: potassium (K_2O) 2-3 grammes; sodium (Na_2O) 4-6 grammes; calcium (CaO) 0.15-0.35 gramme; magnesium (MgO) 0.2-0.3 gramme; iron, traces; chlorine, 6-8 grammes; phosphoric acid (P_2O_5) 2.0-3.5 grammes. The total mineral (ash) in 24 hours varies from 9 to 25 grammes.

The Quantitative Determination of the Total Mineral Constituents (Urinary Ash).—Evaporate to dryness from 25 to 50 c.c. of the 24 hours' urine and carbonize the residue slowly at a moderate temperature in order not either to drive off the sodium and potassium chlorides, reduce the sulphates or decompose the phosphates. After carbonizing treat with water; pour off the aqueous extract, saving it; dry the residue over the water-bath, and finally incinerate. Evaporate the watery extract and incinerate in the same crucible. Weigh the ash, and from its weight calculate the total weight in 24 hours.

COMPOUNDS OF HYDROCHLORIC ACID (CHLORIDES).

Next to nitrogen common salt is the principal constituent of the urine, the chlorides forming nearly one-quarter by weight of the total solids eliminated by way of the urine.

Chemistry.—Sodium chloride, NaCl, is the principal compound of hydrochloric acid. In smaller amount chlorides of potassium, ammonium and magnesium occur. The chlorides are always in solution and do not occur in the sediment nor in calculi.

Physiology.—The source is the food, since the fixed tissues contain little or no chloride. Normally all the salt taken in the food is excreted in the urine, but the amount varies with the volume of the urine. Nevertheless, the tissues demand a certain quantity of salt, for in starvation the chlorides almost disappear from the urine, and later, when salt-containing food is given, a certain amount of salt is retained in the body daily for a time, until the tissues appear to have enough.

The *amount* of the chlorides in 24 hours, reckoned as chlorine, ranges from 6 to 10 grammes (93-115 grains), as sodium chloride

from 10 to 15 grammes (155-250 grains). Those fond of salt may void as much as 40 to 50 grammes, it is claimed.

Physiological Variations in Amount.—The chlorides are *increased* by the following diet rich in salt: salt pork, corned beef, salt fish, sea foods, mineral waters rich in salt; hearty eating, hence in winter and in day time more than at night; circumstances increasing the circulating protein; active exercise, massage, copious draughts of water (previous retention by affinity for proteins); certain drugs; potassium salts, especially the neutral phosphate, some diuretics, as digitalis, chloroform, thyroid preparations, and possibly salicylic acid.

The chlorides are *decreased* by the following: diet poor in salt, milk diet, potatoes and rice; starvation; distilled water; warm weather, (except when salted pretzels and beer are taken); repose and during the night; following an increase of the circulating albumin (retention for purposes of protein metabolism); temporarily after administration of salicylic acid.

Pathological Variations in Quantity.—The chlorides are *increased* by the following: after the crisis of the acute infections; after the rapid absorption of exudates or transudates, dropsy, etc.; epilepsy, after the attack; *diabetes mellitus* and insipidus (polyuria); cirrhosis of the liver; destruction of the red blood corpuscles; malaria; poisoning by pyrogallic acid, etc.; prurigo; first stage of general paralysis (excessive eating).

The chlorides are *decreased* by the following: chronic lead poisoning; during the formation of transudates and exudates; *pneumonia, pericarditis,* acute exudative and infectious diseases; loss of fluids by diarrhea and vomiting; during formation of dropsy; cholera; pyemia; puerperal fever; acute articular rheumatism; *most chronic diseases* (diminished absorption, light diet, and renal insufficiency) especially of the stomach and kidneys; inanition, anemia, cachexia; impetigo, pemphigus foliaceus; melancholia; idiocy.

☞ **Clinical Notes.**—Definite retention of chlorides occurs in a remarkable way during fevers, especially toward the crisis. Entire absence may be noted in *pneumonia,* and is a serious sign. After convalescence has begun there may be a sudden return

to normal (chlorine crisis). Persistent decrease (0.05 gramme per 24 hours) or absence of chlorides for several days is of bad prognostic significance.

In cases of pneumonia in which the fall in temperature is succeeded by several days of very slight fever, the chlorides may not rise until the temperature becomes normal.

In differentiating between *typhoid* and *meningitis* it is said that the chlorides are moderately low in the former and very low in the latter.

After *surgical operations,* according to Guyon, patients with less than 1 gramme of chloride per liter are not likely to survive.

☞ In *any chronic diseases* a low figure of chlorides is of bad prognostic import, and if the excretion is as low as 2 grammes per 24 hours when the diet contains salt it is usually a sign of approaching death.

The term "chloruremia" has been applied to the condition characterized by partial renal insufficiency for chlorine elimination, low chloride excretion, rapidly developing general edema, and increased albuminuria.

Hence if the rectal injection of normal *salt solution* in nephritis causes increased albuminuria, slight hematuria and uremic phenomena, it can not be deemed as harmless as claimed.

☞ In *gastric disorders* the ratio of urea to chlorides is of diagnostic importance: decrease in both in like proportion indicates merely inanition, whereas decrease in chloride with relatively large amount of urea or nitrogen (more than 2 urea to 1 chloride), may indicate presence of cancer (much tissue-albumin destroyed containing more protein than chlorine).

In *acute rheumatism* the disappearance of chlorides without increased joint involvement suggests pericarditis with effusion.

☞ **Therapeutic Hint.**—Withdrawal of chlorides from the diet is followed in some patients by disappearance of nephritic edema.

Medico-Legal Note.—In cases of malingering, as when persons claim inanition or to be suffering from starvation, the finding of several grammes of sodium chloride in the 24 hours' urine indicates that food was taken on the day preceding, since the body quickly begins to retain salt when food is withdrawn.

CLINICAL TESTS FOR CHLORIDES.

Silver nitrate precipitates the hydrochloric acid as silver chloride, insoluble in nitric acid, hence in any urine acidulated with nitric acid to prevent precipitation of phosphates and coloring matter by silver nitrate, the latter in solution serves to detect the chlorides, a white curdy precipitate being formed when the percentage is normal. Since more or less of the chlorides is present in all urine, except in certain diseases above mentioned, the quantitative determination, as in the case of urea, is clinically of more importance. All the quantitative methods used depend in principle upon the precipitation of silver chloride on addition of silver nitrate to acidulated urine.

In all cases albumin should be removed, if present, by boiling, cooling and filtering the urine clear.

☞ **Rough Estimation by the "Ball" Precipitate.**—To 10 or 15 c.c. of urine, which contains no albumin or from which albumin has been removed, add 10 drops of pure nitric acid; mix well, and add just one drop of solution of silver nitrate, in strength about 10 per cent. If the chlorides are normal or increased, the precipitate sinks as a compact ball. According to the appearance of the precipitate, more or less compact, the quantity of chlorides can be guessed at, a cloudiness without flakes indicating 0.1 per cent. or less. Exposed to the light the precipitate darkens.

Quantitative Determination of Chlorides.—The determination of the quantity of the chlorides may be made either with or without the use of the centrifugal machine. The writer uses volumetric analysis, method of Volhard modified by Luetke, but the centrifugal method of Purdy is popular.

☞ 1. **The Purdy Centrifugal Method.**—This is at best only more or less approximate, as the various percentage tubes sold as Purdy tubes give widely differing results. It should not be used except by those who are unable to make the determinations described below. Care should be taken to *use the same tube or set of tubes* in following the chloride curve in a given case.

Fill the percentage tube (Fig. 12) to the 10 c.c. mark with

filtered albumin-free urine; add 15 to 30 drops of pure nitric acid according to the specific gravity of the urine, 30 drops being necessary in urines above 1025; and fill up to the 15 c.c. mark with the 1:8 solution of silver nitrate recommended by Dr. Purdy, made according to English weights, one drachm to the fluidounce of distilled water; cork the tube; mix well and place in the Purdy electric centrifuge with a companion tube filled with water. Sediment at the rate of 1500 revolutions for five minutes; stop the machine for an instant; then sediment again for five minutes, and once again. Read off the bulk percentage. In reading the percentage note that the figure $\frac{1}{2}$ indicates 5 per cent. by bulk, the figure 1, 10 per cent., etc.; hence every $\frac{1}{10}$ c.c. represents 1 per cent. by bulk.

The average normal bulk percentage is 10, the normal range from 4 to 12 per cent. indicating from 0.5 to 1 per cent. of sodium chloride, or from 7.5 to 15 grammes in 24 hours' urine, measuring 1500 c.c.

By this method, during the crisis in pneumonia, care must be taken to remove all proteins in the urine, since silver nitrate produces a flocculent precipitate from mucus or other proteins, which should not be measured as chloride. The chloride precipitate is dense, compact, and at first white. After this compact white precipitate disappears in pneumonia, the flocculent precipitate mentioned above may be noticed and sometimes even after boiling and filtration.

☞ 2. **Quantitative Determination With Silver Nitrate and Chromate Indicator.**—This method is extremely simple and in most cases sufficiently accurate for clinical purposes. A solution of silver nitrate is made containing 29.075 grammes in 1000 c.c. of water, and a burette is filled with this solution. Measure off the 10 c.c. of the urine from which albumin has been removed; dilute with 50 c.c. of water, and add to it 8 or 10 drops of a *saturated* solution of yellow neutral, chlorine-free potassium chromate. Care must be taken that ***not even a trace*** of mineral acid be present in the containers used. Run in the silver nitrate from the burette until a permanent orange replaces the yellow precipitate first formed. Read off the amount of silver nitrate

used and subtract 1 for correction needed on account of influence of purine bodies and coloring matters. The solution of silver nitrate used contains silver nitrate such that 1 c.c. will precipitate 0.01 gramme of sodium chloride; hence the number of c.c. of silver nitrate used indicates grammes per liter of common salt in the urine. For example, if 12 c.c. of silver nitrate were used, then in 10 c.c. of this urine there was 0.01 times 11, or 0.11 gramme sodium chloride; in 100 c.c. 1.1 grammes (1.1 per cent.), and in 1000 c.c. 11 grammes (11 grammes per liter).

From the percentage we may obtain grains per fluidounce by multiplying by 4.55; in the above case 4.55 times 1.1 = 5 grains per fluidounce.

In urines free from albumin, and of specific gravity below 1020, the above method compares closely with the standard volumetric methods described below.

For accurate work the method of Volhard or some modification of it is advised.

3. **The Volhard Method.**—The best methods are Arnold's or Luetke's modifications of the original Volhard. The author favors Luetke's because of the advantage of combining three solutions in one.

Arnold's modification is as follows: make up the following solutions:

(1) Silver nitrate 29.075 grammes of the crystals in enough distilled water to make 1 liter.

(2) Pure colorless nitric acid, of specific gravity 1.20.

(3) A cold saturated solution of ferric alum, about 10 per cent.

(4) An ammonium thiocyanate (sulphocyanate) solution of strength such that 10 c.c. will equal 10 c.c. of the silver nitrate solution.

First make up the silver nitrate solution as follows: test the purity of the crystals by dissolving about 1 gramme in distilled water, heating to boiling, precipitating with dilute hydrochloric acid, and filtering. Evaporate the filtrate in a platinum crucible, and if the substance is pure, practically no residue will be obtained. In case it is not pure the silver nitrate should be re-

crystallized. If pure, dissolve 29.075 grammes in 900 c.c. of water, and standardize with sodium chloride.

To standardize the solution weigh off 0.15 of sodium chloride previously dried by careful heating in a platinum crucible; dissolve in a little distilled water, and further dilute to 100 c.c. To this solution a few drops of a saturated solution of chromate of potassium are added and the mixture titrated with that of the silver nitrate. The nitrate of silver will first precipitate every trace of sodium chloride present, and then combine with the potassium chromate, forming red silver chromate. The slightest orange tinge remaining after stirring indicates the end of the reaction. Were the solution of silver nitrate of the proper strength exactly, 15 c.c. should be used, as every c.c. is to represent 0.01 gm. of sodium chloride. As a matter of fact, less will in all probability be needed, the solution having been purposely made too strong. Its correction then becomes a simple matter, it merely being necessary to determine the degree of dilution required. Supposing that 29.075 grammes of silver nitrate have been dissolved in 900 c.c. of water, and that 14.5 c.c. instead of 15 c.c. had been required to precipitate the 0.15 gm. of sodium chloride, it is evident that every 14.5 c.c. of the remaining solution must be diluted with 0.5 c.c. of water. It is hence only necessary to divide the number of c.c. of the silver nitrate solution remaining by 14.5; the result multiplied by 0.5 represents the amount of water which must be added in order to bring the solution to the required strength.

Hence the rule for the correction of a solution found too strong:

$$C = \frac{N.\ d}{n}$$

in which "C" represents the number of c.c. of water which must be added to the solution remaining; "N," the number of c.c. of the solution on hand; "n," the number actually used in one titration, and "d," the difference between the number of c.c. theoretically required and that actually used in one titration.

In a case where 936.5 c.c. of the solution were on hand the equation would then read:

$$C = \frac{936.5 \times 0.5}{14.5} = 32.29$$

32.29 c.c. of distilled water then are to be added to the 936.5 c.c. on hand and the strength of the solution to be tested by a second titration. If the solution be found too weak, it is best to make it too strong and then to correct as described.

To prepare the ammonium sulphocyanate solution, proceed as follows: 12.90 grammes of the sulphocyanate are weighed and dissolved in a little less than one liter of water, and the solution is poured from one flask to another until well mixed. Fill a clean burette with this solution. Next prepare a mixture of 20 c.c. of the silver nitrate solution, 5 c.c. of the ferric alum, and 4 c.c. of the nitric acid, in a flask, and dilute to 100 c.c. with water, mixing well. Run in the sulphocyanate solution from the burette into the flask until a permanent brown precipitate appears, replacing first the temporary brown and then the white. Dilute the sulphocyanate according to the formula for correction as above, until 20 c.c. of it correspond to 20 c.c. of the silver nitrate. Boston advises weakening the sulphocyanate so that 25 c.c. correspond to 10 c.c. of the silver solution, and computes results accordingly.

The brown precipitate obtained in the end-reaction is due to the formation of ferric cyanate, the iron in the ferric alum uniting with the cyanate when all the silver has been precipitated. Having made up the solution, proceed to determine the chlorides in the urine as follows: 10 c.c. of urine are carefully measured with a pipette into a flask on the neck of which is a 100 c.c. mark. Then are added 20 to 30 drops of nitric acid and 2 c.c. of the iron alum solution. If necessary a few drops of 8 per cent. potassium permanganate are added until all red color disappears. The silver nitrate solution is then slowly run in, constantly shaking the flask until one is sure that all the chlorine has been precipitated and that there is an excess of silver. The flask is then allowed to stand for about ten minutes and then filled to the 100 c.c. mark with water. This should then be mixed very thoroughly. There should be an excess of iron, otherwise the nitric acid can de-

colorize the ferric cyanate; but this excess of iron causes a brown rather than a red color in the reaction.

After the observer is sure that the contents of the 100 c.c. flask are thoroughly mixed, the liquid is then filtered through a dry filter until 50 c.c. of clear filtrate are obtained. This is titrated with the ammonium sulphocyanate solution until the reaction. The amount used indicates the excess of the silver solution in 50 c.c. of filtrate. This amount, multiplied by 2, (since only one-half of the filtrate was used), and subtracted from the number of c.c. of silver nitrate originally added, will give the number of c.c. of silver nitrate actually precipitated by the chlorides in the urine. This figure multiplied by 10 milligrammes will give the weight of the sodium chloride in the amount of urine used.

In adding silver nitrate, from 15 to 20 c.c. are necessary to insure excess. It is probable that 20 c.c. gives the best results.

Example: suppose 8 c.c. of ammonium sulphocyanate necessary to produce the end-reaction, and 20 c.c. of silver nitrate originally added. Then 20 minus 16 $(8 \times 2) = 4$, that is, 4 c.c. of silver nitrate were actually precipitated by the chlorides in the urine. Then 4 times $10 = 40$ milligrammes sodium chloride in 10 c.c. of urine, or 400 milligrammes in 100 c.c. $= 0.4$ gm. in 100 c.c. $= 0.4$ per cent., or 4 grammes per liter. Multiplying 0.4 by $4.55 = 1.82$ grains of sodium chloride per fluidounce.

☞ 4. **Luetke Method.**—Make up a 25 per cent. solution of pure nitric acid, of specific gravity 1.42, by mixing 176 c.c. with 750 c.c. of distilled water. Dissolve 17.50 grammes of pure silver nitrate in 900 c.c. of this nitric acid solution. To this add 50 c.c. of a ten per cent. ferric alum solution and make up the whole to a liter. Make up a tenth-normal ammonium sulphocyanate solution by dissolving 7.6 grammes of the salt in a little less than a liter of water. Fill a burette with the sulphocyanate solution and run it into 10 c.c. of the silver nitrate solution in order to determine its strength. Dilute the sulphocyanate solution according to the formula for correction until 10 c.c. of the sulphocyanate exactly precipitates 10 c.c. of the silver nitrate, shown by the persistence of the brown ferric cyanate.

Measure out 10 c.c. of urine; add 25 c.c. of the silver nitrate solution, a little at a time, with shaking; let stand 10 minutes; dilute to 100 c.c.; mix thoroughly; filter into a dry flask; measure off 50 c.c., and titrate with the ammonium sulphocyanate.

Multiply the number of c.c. of sulphocyanate used by 2; subtract from 25, and multiply remainder by 0.00585, since 1 c.c. of the tenth normal silver solution actually precipitated by the chloride corresponds to 0.00585 grammes of sodium chloride.

The methods above described are expensive, owing to the amount of silver nitrate required to insure an excess. Hence the author modifies the Luetke method as follows: instead of using 10 c.c. of urine mix 5 c.c. of urine with 5 c.c. of water; add to the mixture 12.5 c.c. of the tenth-normal silver nitrate solution, a little at a time, with constant shaking. Let stand 10 minutes, fill up to 100 c.c. with water; mix well; filter dry, and titrate 50 c.c. of the filtrate with the ammonium sulphocyanate. Multiply the number of c.c. of sulphocyanate used by 4; subtract from 25, and multiply this remainder by 0.00585.

The figuring required is tedious, hence the author has prepared a table (see page 108) which shows at a glance the amount of sodium chloride in grammes per liter indicated by a given amount of ammonium sulphocyanate, as follows, using 5 c.c. of urine diluted with 5 c.c. of water.

To obtain the per cent. of sodium chloride, divide the figures in the right hand column by 10 and to obtain the grains per fluid-ounce multiply the per cent. by 4.55.

☞ **Laboratory Note.**—In spite of the fact that some writers claim these methods to be accurate in the presence of albumin in the urine the writer finds that it is best to remove albumin when in any considerable quantity, by boiling, cooling and filtration. It is advisable not to acidulate the urine too strongly, but to make it feebly acid only, adding 20 per cent. acetic acid drop by drop after boiling.

Again, the author finds it unnecessary to wash the albuminous residue on the filter or to add titration of the washings to the figures obtained from the filtrate. The loss of chlorides in the albuminous coagulum in such urines as have been examined by the writer is so small as to be negligible.

NO. OF C.C. OF SULPHOCYANATE USED.	GRAMMES PER LITER OF SODIUM CHLORIDE (5 C.C. OF URINE AND 5 C.C. OF WATER USED INSTEAD OF 10 C.C. OF URINE).	NO. OF C.C. OF SULPHOCYANATE USED.	GRAMMES PER LITER OF SODIUM CHLORIDE (5 C.C. OF URINE AND 5 C.C. OF WATER USED INSTEAD OF 10 C.C. OF URINE).
0.1	14.39	3.30	6.90
0.2	14.15	3.4	6.67
0.3	13.91	3.5	6.44
0.4	13.64	3.6	6.20
0.5	13.46	3.7	5.97
0.6	13.22	3.8	5.73
0.7	12.98	3.9	5.50
0.8	12.75	4.0	5.27
0.9	12.51	4.1	5.03
1.00	12.29	4.2	4.80
1.10	12.05	4.3	4.56
1.30	11.58	4.4	4.33
1.40	11.35	4.5	4.10
1.50	11.11	4.6	3.86
1.60	10.88	4.7	3.62
1.70	10.65	4.8	3.49
1.80	10.41	4.9	3.16
1.90	10.17	5.0	2.92
2.00	9.94	5.1	2.69
2.10	9.71	5.2	2.46
2.20	9.47	5.3	2.23
2.30	9.24	5.4	1.99
2.40	9.01	5.5	1.76
2.50	8.78	5.6	1.52
2.60	8.54	5.7	1.29
2.70	8.31	5.8	1.05
2.80	8.07	5.9	0.82
2.90	7.84	6.0	0.59
3.00	7.60	6.1	0.35
3.10	7.37	6.2	0.12
3.20	7.14		

The difference in chloride determination which albumin makes is shown by the following in a sample of urine measuring 1100 c.c. in 24 hours, of specific gravity 1016, containing 12.65 grammes of urea and 0.175 per cent. by weight of albumin; the Luetke process showed 9.94 grammes per liter of "chlorides" in the original urine and 8.78 in the boiled and filtered urine.

The Dehn-Clark Method.—This is given by Hawk as desirable, in that organic compounds which hold the chlorine too firmly for quantitative precipitation with silver nitrate are destroyed by oxidation with sodium peroxide. To 10 c.c. of urine in a dish of 100 c.c. capacity, add from 1 to 1.2 grammes of sodium peroxide, and evaporate the mixture to dryness on the boiling water-bath, adding more peroxide and evaporating if the residue is not pure white after first moistening with distilled water. Add 10-20 c.c. of distilled water and stir until all has been brought into solution. Drop in a piece of blue litmus paper and add dilute nitric acid (1 : 1) until effervescence ceases and the litmus paper turns red. Heat the dish on a wire gauze until the contents almost boil, and if any precipitate be seen, filter, save the filtrate, and heat it again. Keep the solution or filtrate hot and add silver nitrate solution (29.06 grammes per liter) in slight excess, which excess is most easily recognized by adding the silver solution with constant stirring to the hot chloride solution, so that the silver chloride formed coagulates and sinks, leaving a clear supernatant fluid. Filter off the silver chloride while the solution is still hot, and wash the precipitate thoroughly with distilled water. To the filtrate add 1 c.c. of a saturated solution of ferric alum and titrate with standard ammonium sulphocyanate solution made as described under the Volhard-Arnold method. Use the same end-point of color in all determinations as that obtained in standardizing. The number of c.c. of the sulphocyanate used, subtracted from the number of c.c. of silver nitrate added as above, equals the number of c.c. of silver nitrate actually used for complete precipitation of the chlorine. Multiplication by 0.010 gives weight in grammes of the sodium chloride in 10 c.c. of urine from which the per cent. and total may be readily calculated. Multiplication by 0.006 instead of 0.010 will give weight of chlorine.

CHAPTER IX.

THE COMPOUNDS OF PHOSPHORIC AND CARBONIC ACIDS; CERTAIN OTHER INORGANIC COMPOUNDS.

Chemistry of the compounds of phosphoric acid: earthy and alkaline phosphates.

Ammonio-magnesium phosphate: chemistry.

Occurrence and solubility of the various phosphates.

Physiology of the phosphates: source, quantity, ratio to urea.

Physiological variations in quantity.

Pathological variations in quantity: effect of diabetes, nervous exhaustion, etc.

Clinical notes on the phosphates in pneumonia, typhoid and tuberculosis.

Clinical notes on phosphaturia and phosphatic diabetes.

Clinical notes on phosphates in nephritis and in Addison's disease.

Clinical notes on ratio of nitrogen to P_2O_5.

Clinical tests for phosphates, earthy and alkaline.

Approximate determination of the phosphates.

How to make the magnesian fluid.

Meaning of a cloudiness seen on boiling non-albuminous urine.

Quantitative determination of the phosphates by Purdy's centrifugal method.

Quantitative volumetric determination of the phosphates by uranium nitrate.

Preparation of the volumetric solutions; technique in full.

Laboratory notes on ferrocyanide and cochineal as indicators.

Quantitative determination of earthy and alkaline phosphates, separately.

Glycerophosphoric acid: physiology, clinical significance, and determination by Sotnitschewsky's process.

Gravimetric determination of the total phosphorus.

Chemistry and physiology of the carbonates.

Clinical test for carbonates.

Laboratory note on foaming of alkaline urine.

Interference of ammonium carbonate with analysis.

Miscellaneous inorganic compounds; silicic acid, hydrogen dioxide, nitrous acid, nitric acid, iron, thiosulphuric acid, hydrogen sulphide, and fluorine.

Iron in urine: relation to diabetes, pernicious anemia, etc.

Clinical test for iron.

The inorganic bases in urine; calcium, magnesium, sodium, etc.
Physiological variations in the quantity of calcium and magnesium.
Pathological variations in the quantity of calcium and magnesium; relation to starvation.
Quantitative determination of calcium and magnesium.
Physiology of sodium and potassium:—variations in quantity.
Pathological variations in the quantity; relation to fevers, etc.
Clinical note on excess of potassium over sodium.
Quantitative determination of potassium and sodium.

THE COMPOUNDS OF PHOSPHORIC ACID (PHOSPHATES).

Phosphoric acid, H_3PO_4, occurs in the urine of human beings in considerable quantity in solution, in the sediment, and as a constituent of calculi.

Chemistry.—Phosphoric acid is combined in the urine with the bases ammonium, potassium, sodium, calcium, and magnesium. We distinguish earthy phosphates (compounds of calcium and magnesium), alkaline phosphates (compounds of sodium and potassium), and triple phosphate (ammonio-magnesium phosphate). The constitution and state of the phosphates vary with the reaction of the urine, in acid urine all phosphates being in solution except traces of acid calcium phosphate ($CaHPO_4 2H_2O$) in the sediment.

In neutral or in alkaline urine amorphous earthy phosphates (normal phosphates of calcium and magnesium) appear in the sediment; in *ammoniacal urine* the same are present with also *crystalline triple phosphate,* also called ammonio-magnesium phosphate, $NH_4MgPO_4.6H_2O$. In acid urine the phosphates are "acid" phosphates, *i. e.*, those containing hydrogen; in alkaline urine, "normal" phosphates, *i. e.*, containing no hydrogen. Neutral phosphates (disodium hydrophosphate) may also occur in neutral urine.

The sodium phosphates are more common than the potassium and the calcium than the magnesium, while the alkaline phosphates exceed the earthy in amount.

The alkaline phosphates (sodium and potassium) are always in solution,—never appear in the sediment nor in calculi; nor are they precipitated by boiling, hence do not complicate the albumin tests.

The earthy phosphates (calcium and magnesium) are present in solution in all normal urine and in the sediment of alkaline urine. Precipitated from solution in feebly acid, neutral or alkaline urine when the latter is boiled. Precipitated whenever an alkali like liquor potassæ is added to the urine, as in the various tests for sugar (Haines' or Fehling's). Redissolved when any acid is added to the precipitate or to the sediment. Triple phosphate is not found in normal urine until after ammoniacal decomposition has set in, but in conditions causing retention of urine, with decomposition of urea and formation of ammonium carbonate, some of the ammonium unites with the magnesium phosphate to form the double salt, ammonio-magnesium phosphate. The term "triple" has been applied to it on account of the $6H_2O$ forming a third part of its formula $NH_4MgPO_4.6H_2O$. Inconplete crystals of it may occur in feebly acid urine and in alkaline urine without odor of ammonia, but in greater amount and more perfect crystalline form it is found in the sediment of ammoniacal urine. (See "Sediments.")

Small quantities of phosphoric acid combined with organic radicals also occur (glycerophosphoric acid).

Physiology.—The source of the phosphates is chiefly the food; to a less extent, tissue metabolism. Bone, meat, cereals, and nuclein-rich articles add to the amount of inorganic phosphates; eggs, milk, etc., contain organic phosphorus (lecithin, nucleoalbumin) which is readily oxidized and more easily assimilated. From the tissues the decomposition of lecithin in brain and nerve tissue and of nuclein in the muscles, brain, milk, seminal fluid, etc., furnishes phosphates. The more the calcium content of the diet the less phosphates in the urine and the more in the feces, owing to the affinity of phosphoric acid for mineral salts.

The *quantity varies*, as may be inferred from the above.

Ten thousand analyses made by the author in Chicago, a limestone locality, shows a normal average below that of the books, namely, from 1.5 to 2.5 grammes per 24 hours. According to Long it is from 1.3 to 3.5 grammes.

Children void from 0.5 to 1.5 grammes.

The ratio of the alkaline phosphates to the earthy is 2 to 1; of

the diacid to the monacid, 6 to 4. The most important phosphate is the diacid sodium salt, NaH_2PO_4. The normal ratio of urea (clinically) to phosphoric acid (P_2O_5) averages 10; ranges 8-12 to 1. Phosphaturic coefficient, P_2O_5 to nitrogen, ranges from 17:100 to 20:100.

Physiological Variations in Amount.—The phosphates are *increased* by meat diet; nuclein-rich diet; just after copious draughts of fluids; hard manual labor; during sleep induced by chloral or potassium bromide; by salicylic acid, and the glycerophosphates. They are *decreased* by vegetarian diet; milk diet; nuclein-poor diet, following copious draughts of fluids; during rest; light eating; and pregnancy; by calcium carbonate, ether, alcohol, cocaine, and quinine.

Pathological Variations in Amount.—The phosphates are increased by the following: Phosphorus poisoning; acute diseases (after the crises); *all forms of diabetes;* meningitis; acute yellow atrophy; cholera infantum; leukemia (advanced stages); pseudoleukemia; small pox; epileptic attacks; bone diseases (disputed); early tuberculosis; nervous diseases (author's experience opposed).

The phosphates are *decreased* in acute diseases before the crisis, especially *pneumonia;* in most chronic diseases, especially *nephritis and Addison's disease;* pyuria; gout; rheumatism; osteomalacia; arthritis; anemia; hysterical attacks; cirrhosis of the liver.

Clinical Notes on Increase of P_2O_5.—A sharp rise in the total phosphate may occur at the crisis in *pneumonia* but not necessarily at the same time with that of the chlorides.

A case of *typhoid* is mentioned by Emerson in which the P_2O_5 rose from 1.5 to 13 grammes in 24 hours after defervescence.

Gouraud thinks the differential diagnosis between *tuberculous processes and pneumonia* may be helped by the determination of the ratio of earthy phosphates to the alkaline, claiming an increase of the earthy in tuberculosis.

In 25 cases of *diabetes mellitus* with polyuria examined by the author the highest excretion of P_2O_5 was 5.8 grammes, and in many hundred other cases no great excess was noted.

☞ The term "*phosphaturia*" is used clinically to denote the condition in which the urine persistently deposits a whitish sediment of *earthy* phosphates, though the total P_2O_5 is not necessarily excessive. In some cases the earthy phosphates are increased in amount, but even this is not true of all. The sediment is due to deficient acidity of the urine, as can be proved by a determination of it, and often by the litmus paper test alone. It is common in *neurasthenia* and *after gonorrhea.* In *hysterical conditions* there may be a relative increase of the earthy phosphates.

"Phosphatic diabetes" is the term applied to a condition in which, without sugar, P_2O_5 is above 3 or 4 grammes per 24 hours. The cases are more likely neurasthenic, with nervous polyuria, or of true diabetes mellitus with a temporary absence of sugar.

Clinical Notes on Decrease of P_2O_5. The author having determined the total phosphate in some 8,000 persons, well or ill, finds a *decrease* in the amount extremely common and an increase comparatively rare.

☞ There is no question about the deficiency in *renal diseases.* Less than 1.5 grammes per 24 hours were found by the author in the majority of 53 fatal cases of subacute and chronic nephritis, with an average of only 1.14 grammes for the whole number.

The total phosphates are likely to be decreased to a greater degree than urea in *nephritis,* hence a marked deficiency in a suspicious case becomes of diagnostic importance.

When the amount of P_2O_5 falls below 1 gramme in *nephritis* the case is a serious one. Out of 41 such cases observed by the author 31 died in a short time.

☞ In *Addison's disease* the ratio of urea to phosphoric acid may be greatly increased, as high as 17-20 to 1 being observed by the author in 2 cases without increase of the total urea per 24 hours. The ratio of nitrogen (urea) to the total P_2O_5 is *increased* by cerebral excitants, as strychnine, alcohol, phosphorus, valerian, cold baths, salt baths; and *decreased* by cerebral depressants, as chloroform, morphine, chloral, alcohol in large quan-

tity, potassium bromide, acids, prolonged cold baths, Turkish baths, and low temperature.

CLINICAL TESTS FOR PHOSPHATES.

The phosphates may be detected and approximately estimated as follows:

☞ **Earthy Phosphates.**— A test-tube 16 centimeters (6.2992 inches) long and 2 centimeters (0.787) wide, is filled *one-third* with clear or filtered urine, to which a few drops of ammonia or caustic alkali solution are added, and warmed gently over a spirit lamp until the earthy phosphates begin to separate in flakes. It is then placed aside for ten to fifteen minutes for them to subside. If the layer of sediment is one centimeter (0.3937 inch) high, the earthy phosphates are present in normal amount; if they occupy two to three centimeters (0.787 to 1.181 inch), they are increased; if, on the other hand, only a few flakes are visible, the earthy phosphates are diminished.

☞ **Alkaline Phosphates.**—Filter the mixture obtained above and to the filtrate add one-third its volume of magnesian fluid. A snow-white precipitate, crystalline *ammonio-magnesium phosphate*, mixed with amorphous calcium phosphate, occurs. (The crystals thus rapidly formed have a fern-leaf shape, not prismatic nor coffin-lid, as in spontaneous deposits. See "Sediments.")

The quantity of alkaline phosphates may be approximately determined by warming and allowing to settle, comparing the bulk of the sediment obtained with that of an average specimen of normal urine. Or, add to 10 c.c. of any sample of urine 3 c.c. of magnesian fluid, and if the urine becomes milky the amount is normal; if creamy, increased; but if only slightly turbid, decreased.

☞ **Laboratory Notes.**—The magnesian fluid used is made by dissolving 56 grammes of magnesium chloride in 400 c.c. of distilled water, adding 70 grammes of ammonium chloride and when solution has taken place further adding 350 c.c. strong ammonia water. After this the solution is diluted with distilled water to make one liter.

The mixture may also be made of magnesium sulphate, ammonium chloride and ammonium hydrate, of each one part by weight, and of water 8 parts.

☞ Heating certain samples of urine may cause a turbidity which is readily dissolved by acetic acid; the flocculent precipitate thus produced is due to calcium phosphate with a trace of oxalate and sulphate. It occurs in urines deficient in acidity and must not be confounded with the appearance of albumin on boiling, the latter when coagulated being insoluble in acetic acid.

QUANTITATIVE DETERMINATION OF THE PHOSPHATES.

1. **Quantitative Determination of the Phosphates by Purdy's Centrifugal Method.**—Fill the Purdy percentage tube to the 10 c.c. mark with the urine; add 2 c.c. of 50 per cent. acetic acid; shake; add 3 c.c. of a 5 per cent. solution of uranium nitrate. Cork the tube; mix well by inverting; let stand three minutes; place in the Purdy centrifuge and cause to revolve at the rate of 1,500 revolutions a minute, for three minutes. The bulk percentage of precipitate corresponds to grammes per liter of P_2O_5, as follows:

1	per	cent.	bulk	= 0.40	grammes	per	liter
5	"	"	"	= 0.80	"	"	"
10	"	"	"	= 1.30	"	"	"
15	"	"	"	= 1.80	"	"	"

that is, each per cent. by bulk ascends by 0.10 gramme per liter; hence 6 per cent. by bulk would indicate 0.90 gramme per liter; 7 per cent. 1.00, etc. Divide by 10 to obtain per cent. and multiply per cent. by 4.55 to obtain grains per ounce. In using the centrifugal method for determination of phosphates care should be taken to obtain the Purdy electric centrifuge, with an arm of 6.75 inches and the properly made and graduated percentage tubes. A set of two tubes which agree closely in percentage readings is essential and if one or the other be lost or broken, it should be replaced with another of exactly the same kind.

So-called Purdy percentage tubes are sold which will vary in percentage reading from those which are properly made and graduated. In case of doubt the readings obtained should be

compared with the results of quantitative determination, by the following method:

☞ 2. **Quantitative Volumetric Determination of the Phosphates.**—The volumetric determination calculates the phosphates as phosphoric anhydride, P_2O_5, by precipitation as uranyl phosphate with uranium (uranyl) nitrate or acetate. A solution of uranium nitrate is made such that 1 c.c. corresponds to 0.005 of P_2O_5, that is, such that 20 c.c. exactly precipitates 50 c.c. of a solution of disodium hydrophosphate, Na_2HPO_4, containing 10.085 grammes in a liter. Since the preparation of the disodium phosphate solution is tedious, the uranium solution may be safely made by dissolving exactly 35.461 grammes of uranium nitrate, $UO_2(NO_3)_26H_2O$, Merck's guaranteed reagent, in water to make one liter.

In addition, (1) a solution of sodium acetate is made containing 100 grammes of the salt and 100 c.c. of 30 per cent. acetic acid in water to make one liter; and, (2) a ten per cent. solution of potassium ferrocyanide to be used as indicator.

Emerson recommends addition of three grammes of sodium acetate to the liter of uranium solution on account of liberation of free nitric acid.

In order to determine the amount of phosphate in the urine, 50 c.c. of urine are measured off and 5 c.c. of the sodium acetate solution well mixed with it in order to neutralize any free nitric acid given off. The mixture is placed in a beaker on the boiling water-bath and the uranium nitrate solution run into it, a few c.c. at a time. Drops of the ferrocyanide solution are placed upon a white plate or filter paper near by in a good light, and from time to time by means of a glass rod a drop from the urine mixture is transferred to a ferrocyanide drop upon the plate. As soon as the slightest tinge of red-brown color appears on the plate, the reaction is over. Care must be taken to wipe off the glass rod with a clean cloth each time before dipping it into the urine again, and the ferrocyanide drops should not be allowed to dry by too long exposure, fresh drops being added if the determination is a long one. The process may usually be shortened by observation of the specific gravity of the urine. That is,

if the urine has a specific gravity of 1015 or upwards it is usually safe to run in at first 10 c.c. of uranium without testing; after each addition of uranium to the urine the mixture should be well stirred with the glass rod.

Since each c.c. of uranium used corresponds to 0.005 of P_2O_5, multiplication of the number of c.c. used by 0.005 will show the amount in 50 c.c. of urine, and this product by 2 the per cent. Much more quickly, however, can the calculation be made by the author's method of dividing the number of c.c. used by 10 to get grammes per liter, or by 100 to obtain percentage. Example: suppose 13 c.c. of uranium nitrate necessary to obtain the reddish brown color on the plate by transferring a drop from the urine-mixture; $13 \div 10 = 1.3$ grammes per liter $= 0.13$ per cent. If there were 1200 c.c. of urine in 24 hours, then 1.2 times $1.3 = 1.56$ grammes P_2O_5 per 24 hours.

☞ **Laboratory Notes.**—Care should be taken, when the ferrocyanide shows a color, to wipe off the glass rod with a clean cloth and test again by transferring another drop to the plate. Repetition of the color shows correctness of the work, which can be still further verified by running in another c.c. of uranium, transferring a drop, and obtaining a color much more marked.

Occasionally the ferrocyanide fails to show a slight color but suddenly gives an intense color. It is well in such cases to subtract 1 c.c. from the number of c.c. of uranium used.

In high-colored biliary urine acidify with hydrochloric acid, decolorize with permanganate, and neutralize with sodium hydroxide.

Certain writers advise the use of cochineal tincture as indicator, made by digesting a few grammes of pulverized cochineal insects in 250 c.c. of 25 per cent. alcohol and filtering after several hours. A few drops are added to the urine and the end-reaction is denoted by formation of a green color. Cochineal is much more convenient to use than ferrocyanide, but unfortunately is unreliable, failing to indicate in certain cases, as, *e. g.*, of hemoglobinuria, even when the ferrocyanide indicator shows a great excess of uranium to have been added.

The ferrocyanide is also faulty as an indicator when ammonium

salts are present; hence use as fresh urine as possible, or neutralize alkaline urine with glacial acetic acid.

3. **Quantitative Determination of Earthy and Alkaline Phosphates.**—Add excess of ammonia water to 100 c.c. of the 24 hours' urine in which the total P_2O_5 has been calculated as above. Let stand 12-24 hours; collect the precipitated earthy phosphates on a filter; wash with dilute ammonia water; wash off the precipitate with hot water into a beaker; dissolve in a little dilute acetic acid: add water to make 50 c.c., then 5 c.c. of the sodium acetate solution, and titrate the P_2O_5 as above. The result multiplied by 0.005 is the P_2O_5 in grammes bound to the earthy phosphates of 100 c.c. of urine. Calculate the total for 24 hours in the usual manner. Determine the amount of P_2O_5 combined with the alkaline phosphates by subtracting the total earthy P_2O_5 from the total P_2O_5 as above.

GLYCERO-PHOSPHORIC ACID.

Glycero-phosphoric Acid, $C_3H_9PO_6$, occurs in small traces in urine, resulting as a decomposition product of *lecithin,* which is a combination of choline and glycero-phosphoric acid, occurring in the bile, brain, yolk of egg, etc.

The normal quantity of glycero-phosphoric acid in urine is said to be about 15 milligrammes per liter. It is *increased* in chyluria, pernicious anemia, dementia, lesions of the brain substance, diabetes mellitus, and after chloroform narcosis.

The amount of glycero-phosphoric acid in the urine is said to be 1 per cent. of the total P_2O_5. The determination (Sotnitschewsky's process) is as follows: render the 24 hours' urine alkaline with milk of lime, and precipitate with calcium chloride. Filter; evaporate the filtrate, and extract the residue with alcohol. Dissolve the insoluble alcoholic residue in water. To both solutions add a solution of ammonia and magnesium hydroxide and allow the mixture to stand 24 hours, in order to remove traces of the inorganic phosphoric acid that may still be present. Filter; render the filtrate strongly acid with sulphuric acid, and boil for some time in order to separate the glycero-phosphoric acid. After cooling add ammonia, and let stand. Crystals of ammonio-magnesium phosphate are deposited.

Filter the liquid; wash the precipitate on the filter with dilute ammonia water (1 :5); dry; incinerate and weigh.

Magnesium pyrophosphate ($Mg_2P_2O_7$) is formed, from which the P_2O_5 combined organically can be reckoned, since the weight of the pyrophosphate found, multiplied by 140.9 and divided by 221.1, will give it, according to the following proportion:

Molecular weight of magnesium pyrophosphate (221.1) is to weight in grammes of pyrophosphate as molecular weight of P_2O_5 (140.9) is to the weight of P_2O_5 in grammes in quantity of urine used.

Gravimetric Determination of the Total Phosphorus.—The determination by the sodium hydroxide and potassium nitrate method is as follows: evaporate 25 c.c. of urine to a syrup on the water-bath in a large silver crucible. To the residue add 10 grammes of sodium hydroxide and 2 grammes of potassium nitrate, and fuse until clear. Cool the mixture; transfer it to a dish by means of hot water; acidify slightly with pure nitric acid, and evaporate to dryness on the water-bath. Moisten the residue with a few drops of weak nitric acid; dissolve in hot water, and transfer to a beaker. Add an equal amount of ammonium molybdate solution [molybdic acid 1 part by weight, ammonia water (0.90) 4 parts, nitric acid (1.42) 15 parts] and keep the mixture at 40° C. (104° F.) for 24 hours. Filter off the precipitate, wash it with weak molybdic solution and dissolve in dilute ammonia. Add dilute hydrochloric acid to the solution but not enough to neutralize the ammonia. Next add 10-15 c.c. of magnesia mixture. (This consists of 175 grammes of magnesium sulphate, 350 grammes of ammonium chloride and 1,400 c.c. of distilled water. Dissolve, and add 700 grammes of strong ammonia water; mix thoroughly, and keep in a glass-stoppered bottle.) Stir thoroughly after adding the magnesia mixture; make strongly alkaline with strong ammonia water, and let stand in a cool place 24 hours. Filter off the precipitate: wash free from chlorine; dry; incinerate; weigh as magnesium pyrophosphate, and calculate P_2O_5 as above.

MISCELLANEOUS INORGANIC CONSTITUENTS.

Among these we find carbonates, silicates, nitrates and nitrites, hydrogen peroxide, thiosulphates, hydrogen sulphide, and fluorides.

Carbonates.—Carbon dioxide occurs in the urine both free and combined. A liter of normal urine of acid reaction and of specific gravity 1020, contains 50 c.c. of carbon dioxide capable of being expelled by a current of air. Neutral or alkaline urine may contain more than 100 c.c. per liter. The possible range is from 17 c.c. in urines of low specific gravity to 294 c.c. in those of high.

Combined, we find the carbonates, alkaline (sodium and potassium) and earthy (calcium and magnesium), in small quantities, 2 to 10 c.c. per 24 hours in terms of carbon dioxide.

Urine freshly voided of alkaline reaction is cloudy when voided from presence of earthy carbonates mixed with phosphates; if not cloudy when voided it soon becomes so on standing. Ammoniacal urine contains ammonium carbonate in solution in considerable amount. Such urine effervesces strongly when acids is added, and has a dung-hill odor. Carbonates are either neutral or acid; the acid carbonates (bicarbonates) of the alkalies are less soluble than the neutral salts, while the earthy bicarbonates are more soluble than the neutral.

The **quantity** of carbonates is **increased** by salts of vegetable acids (malic, tartaric, lactic, succinic, etc.) which form carbonates by oxidation; hence a vegetarian diet favors an increase.

Clinical Test.—Addition of an acid causes bubbles of gas (CO_2) which, if set free in a hydrogen-gas apparatus and passed through tubing into lime-water, causes formation of a cloudy precipitate,—calcium carbonate, readily dissolved by acids.

Laboratory Note.—The foaming of alkaline urine when acid is added to it, as in testing for albumin, indicates the presence of an increased amount of carbonic acid; if in addition there is an ammoniacal odor, the presence of ammonium carbonate in large amount is shown. Such urine is difficult to handle for purposes of quantitative analysis, since some of the urea has been converted into ammonium carbonate, and both the Folin method for

uric acid and the uranium process for phosphoric acid are rendered inaccurate. Even the freshly voided urine in certain cases of cystitis and nephritis may contain so much ammonium carbonate as to render accurate quantitative analysis difficult or impossible.

Silicic acid, hydrogen peroxide, nitric and nitrous acids, iron, thio-sulphuric acid, hydrogen sulphide and fluorine have been found in the urine by various observers, but are of little interest. Hydrogen sulphide may be noticed by its color in decomposing urine containing pus. Such urine blackens lead paper if acid or acidulated. Occasionally sulphuretted hydrogen occurs in the urine, due to the existence of a fistulous communication between the rectum and the bladder.

Iron in the urine is of interest clinically owing to the amount present in fevers, sub-acute nephritis, malaria, anemia, pernicious anemia, chlorosis, leukemia, alcoholism and diabetes mellitus. It occurs in organic combination and normally is found only in traces, the bulk of iron compound ingested appearing in the feces. In pernicious anemia the increase in iron is due to the rapid destruction of hemoglobin. Normally the amount varies from 1 to 10 milligrammes in 24 hours. As much as 16 milligrammes have been found in malaria, pernicious anemia, etc.

In diabetes the excretion of iron is parallel to that of sugar.

Clinical Test.—(1) Iron is detected in urine as follows: evaporate 10 to 15 c.c. of urine to dryness over the water-bath. Incinerate. Dissolve the residue in pure hydrochloric acid free from iron and dilute with 5 c.c. of water. Divide the solution into two parts and test with ammonium sulphocyanate and potassium ferrocyanide respectively, a red color and a blue being obtained. The quantity of the iron present may be inferred by the depth of the colors obtained on diluting with water, after the colors have been formed. (2) Neumann's method for the **quantitative determination** of the urinary iron is as follows: prepare four solutions, the first one of ferric chloride containing 0.02 gm. iron wire dissolved in 2 c.c. of hydrochloric acid (sp. gr. 1.19); second, a sodium thiosulphate solution containing one gramme of this salt and one gramme of ammonium carbonate

per liter; third, a solution of starch (one gramme in half a liter of water boiled for 10 minutes). Fourth, a zinc solution containing 25 grammes of iron free zinc sulphate and 100 grammes of sodium phosphate each separately dissolved in water and mixed; the resulting zinc phosphate is cautiously dissolved in dilute sulphuric acid and the solution made up to a liter.

The thiosulphate solution should be frequently titrated as follows: 10 c.c. of the ferric chloride solution mixed with water, a few c.c. of the starch solution and about 1 gramme of potassium iodide are warmed to 60° C. (140° F.) and titrated with the thiosulphate until the blue passes into violet and then just disappears. In this case the number of c.c. of thiosulphate used corresponds to 2 mg. of iron.

In order to determine the iron in urine add to 500 c.c. of urine 50 c.c. of strong sulphuric acid and drop the mixture into a Jena flask containing 30 c.c. of nitric acid at such a rate that when the fluid boils strongly the volume in the flask is not over 100 c.c. Concentrate the whole to 50 c.c., add 10 c.c. of a mixture of equal parts strong sulphuric and nitric acids, and gently heat the whole until a faint yellow appears. Three volumes of water are then added and all is boiled for ten minutes.

A brown vapor is given off due to the decomposition of the nitrosyl-sulphuric acid. Add and mix 20 c.c. of the zinc reagent and after cooling add ammonia water until a permanent white precipitate is obtained. Add a slight excess of ammonia to dissolve the precipitate and heat the fluid to boiling. Separate the crystalline deposit by decantation and pass the hot fluid through a small filter. Test the filtrate with hydrochloric acid and potassium sulphocyanate. If a strong red color is obtained, pour back the filtrate and heat again. If the iron is small in amount add 10 c.c. of the standard iron solution and remember to subtract 2 mg. from the final result. Wash the precipitate in the flask three times with hot water, decant off excess, test wash-water with a crystal of potassium iodide, some starch, and one drop of hydrochloric acid, absence of violet color insuring absence of nitrous acid. Then transfer the precipitate to a flask, dissolve in a little hot hydrochloric acid and titrate with the thiosulphate as above described.

THE INORGANIC BASES.

These are calcium, magnesium, sodium, and potassium. *Calcium* and *magnesium* are excreted as mono-phosphates chiefly, CaH_2PO_4. In neutral or slightly acid urine some of the calcium is probably excreted as a di-phosphate, Ca_2HPO_4. The total phosphates of the earths amount to about 1 gramme per 24 hours, of which calcium, weighed as CaO, ranges from 0.12 to 0.25 gramme, and magnesium from 0.18 to 0.28 gramme. Most of the calcium taken into the stomach appears in the feces, a small percentage (5-10) only being found in the urine. Even if injected subcutaneously, most of it is found in the feces. Magnesium is absorbed more easily and appears, therefore, in larger proportion in the urine, the amount of the former being about twice as much as that of the calcium. The source of both is the food and water ingested.

Physiological Variations in Quantity.—Calcium and magnesium in the urine are increased by a diet of milk and eggs, hard water, and muscular exercise. They are decreased by an animal diet and by soft water.

Pathological Variations.—Calcium and magnesium are increased by starvation, inanition, debility, and wasting generally; by acidosis, as in diabetes mellitus; by febrile diseases and tuberculosis. They are decreased by alkaline medication.

Quantitative Determination of Calcium and Magnesium.—Two hundred c.c. of filtered urine are made alkaline with ammonia water until there is a distinct precipitate. This is then redissolved by adding a little hydrochloric acid, the least amount necessary to effect solution; solid sodium acetate is then added (in amount sufficient to cause a distinct odor of acetic acid to be noticed) and excess of a saturated solution of ammonium oxalate. The mixture is then allowed to stand in warm place for 12 hours. The precipitate that forms is collected on a small filter of known ash-weight, and washed free from chlorides by repeated decantations with boiling water. The filtrate must give no cloud with silver nitrate.

The filtrate and washings should be kept for determination

of the magnesium, a little thymol being added to prevent bacterial fermentation. The filter holding the calcium oxalate is dried at 100° C. (212° F.) and transferred to a weighed platinum crucible, incinerated for a long time over a small flame until it is white on cooling, and then glowed to a white heat for ten minutes over a blast flame; allowed to cool in a desiccator and repeatedly weighed until the weight is constant. The difference between this weight, when constant, and that of the platinum crucible indicates the amount of CaO, one part of which represents 1.845 of calcium phosphate.

Magnesium.—The filtrate and washings from the calcium oxalate precipitate are treated with $\frac{1}{3}$ volume of 10 per cent. ammonia (sp. gr. 0.90); this precipitates all of the magnesia as ammonium-magnesium phosphate. The precipitate is allowed to settle for two or three hours and is then gathered on a filter of known ash-weight and repeatedly washed with a mixture of $\frac{1}{3}$ ammonia and $\frac{2}{3}$ water.

The filter is thoroughly dried, shaken into a platinum crucible, the paper burned in a platinum spiral and its ash added to the crucible, the whole being there fused for fifteen minutes. On account of uric acid it is well to cool and add a small piece of ammonium nitrate in a little water, warm slowly and finally burn this, obtaining a pure white ash.

The result is magnesium pyrophosphate, $Mg_2P_2O_7$, derived from incineration of the ammonio-magnesium phosphate. One hundred parts by weight equal 36.208 parts of MgO.

Volumetrically the amount of calcium may be determined after incineration by transferring in the state of mixed oxide and carbonate to a flask by means of the wash-bottle, and adding an excess of decinormal nitric acid with a graduated pipette. The amount of acid over and above what is necessary to neutralize the mixture can then be titrated by decinormal alkali; each c.c of acid used for saturation represents 0.0028 gramme of calcium oxide, CaO.

Magnesium may be volumetrically determined by dissolving the washed precipitate of ammonio-magnesium phosphate in acetic acid and titrating with the uranium nitrate solution used

for phosphates. Each c.c. of uranium nitrate solution used is equivalent to 0.002815 gramme of MgO.

Laboratory Notes.—The CaO may be calculated from calcium carbonate by converting the incinerated mass into carbonate by repeated treatment with concentrated ammonium carbonate solution, slowly drying and gently igniting until a constant weight is obtained. One part of calcium carbonate by weight equals 0.40 parts of Ca and 0.56 parts of CaO.

The calcium may be determined also as sulphate after burning the precipitate of oxalate white with the blast flame, adding concentrated solution of ammonium sulphate, burning again, and so on until there is no increase in weight. One part of $CaSO_4$ equals 0.41176 CaO.

SODIUM AND POTASSIUM.

These bases are excreted in combination with the various acids, hydrochloric, phosphoric, and sulphuric, in amount (reckoned as oxides) 6.50 to 11.30 grammes per 24 hours, the usual ratio of sodium to potassium ranging from 3:2 up to 5:3; hence we find from 4 to 8 of sodium (Na_2O) and 2 to 4 of potassium (K_2O). The excess of sodium is largely due to the craving for sodium chloride.

Physiological Variations in Quantity.—The amount of both sodium and potassium depends upon the food: *potassium* is *increased* by meat, vegetable, or cereal diet, by sodium citrate and phosphate, by excessive exercise, and by starvation. *Sodium* is *increased* by a mixed diet with plenty of common salt, by hearty eating, and by ingestion of potassium citrate and phosphate.

Pathological Variations.—An *increase* of *potassium* is observed in fevers and all diseases in which there is tissue destruction. *Sodium* is *increased* in convalescence from fevers and in tissue reconstruction.

Clinical Note.—The excess of potassium salts over those of sodium in fevers or starvation is significant of the care which the body takes to preserve its stock of sodium chloride.

Quantitative Determination of Sodium and Potassium.—Among

the many methods described for the determination of sodium and potassium a convenient one is that which converts all the salts of these elements into chlorides, isolates them as such, determines the potassium as double chloride of platinum, calculates the potassium as chloride and subtracts from the total chloride to obtain the sodium, as follows: one hundred c.c. of urine are precipitated fully by baryta water and evaporated to dryness, the dry residue incinerated at red heat, and carbonaceous residue extracted with hot water and repeatedly washed with water on a filter. The filtrate is evaporated to dryness and redissolved in hot water; this causes a precipitation of earthy salts which are filtered off; the filtrate is evaporated to dryness with hydrochloric acid, and the residue of alkaline chlorides glowed at a low temperature and weighed. The residue is now dissolved in a little water, poured into a platinum crucible and treated with an excess of platinum chloride solution; the liquid is slowly evaporated on a water-bath until a crystalline sediment begins to form on cooling. After crystallization is complete, a mixture of one part of ether and four parts of absolute alcohol is poured into the dish and the mixture allowed to stand for two hours. The supernatant liquid is poured off through a small weighed filter, the crystals of potassium-platinum chloride then washed on the filter with the ether-alcohol mixture and repeatedly washed with the same liquid. The filter is then dried in a vacuum desiccator and weighed. This weight less the weight of the filter indicates the amount of potassium-platinum chloride.

The amount of potassium chloride is calculated from the double salt, since 100 parts of potassium-platinum chloride correspond to 30.69 parts of potassium chloride. The amount of the total chlorides obtained above, less the amount of potassium chloride, gives the amount of sodium chloride. To reckon the potassium as K_2O multiply the chloride amount by 0.6317; to reckon the sodium as Na_2O, multiply the chloride by 0.5302.

CHAPTER X.

THE COMPOUNDS OF SULPHUR, INORGANIC AND ORGANIC.

Oxidized and unoxidized sulphur.
The two forms of oxidized sulphur;—preformed and ethereal sulphates.
Chemistry of the inorganic oxidized sulphur;—the sulphates of sodium and potassium.
Chemistry of organic oxidized sulphur;—the conjugate sulphates.
The chemistry of indoxyl: relation to indole.
The change from indoxyl to indigo-blue graphically represented.
Chemistry of skatoxyl, indigo-red, phenyl and cresyl compounds, hydrochinon and catechol.
The term "acid sulphates."
Composition of the unoxidized sulphur:—cystine, etc.
Composition of taurocarbaminic acid, cystine, cysteine, etc.
Physiology of the oxidized sulphur, inorganic and organic.
Physiology of "indican," skatoxyl, phenyl, and cresyl compounds.
Physiology of the unoxidized sulphur.
Total quantity of sulphur compounds.
Total quantity of preformed sulphates and ratio to nitrogen.
Physiological variations in quantity of inorganic sulphates.
Physiological variations in quantity of organic sulphates.
The phenol excretion separately
The quantity of unoxidized sulphur and variations therein.
Pathological variations in quantity: effect of fever, etc., on preformed sulphates.
Pathological variations in quantity: effect of protein putrefaction on ethereal (organic) sulphates.
Clinical notes on the ethereal sulphates in bowel diseases.
Clinical notes on the relation of indicanuria to albuminuria.
Clinical notes on the relation of indicanuria to many diseases.
Relation of indicanuria to the fruit season and to storage eggs.
Diagnostic points on indicanuria: origin or cause.
Use of the sinusoidal current in diagnosis of indicanuria.
Pathological variations in the quantity of neutral (unoxidized) sulphates.
Clinical tests for the preformed (inorganic) sulphates.
Approximate determination of preformed (inorganic) sulphates.
Clinical tests for ethereal sulphates as a whole.

Clinical tests for Indican (Jaffé's, Obermayer's).
Author's modification by treatment beforehand with lead solution.
Askenstedt's test in full, from original paper by him.
Daland's test in full, from original paper by him.
Oxidising agents employed by different analysts.
Indican reaction in presence of iodides.
Clinical tests for skatoxyl: Rosenbach's reaction.
Isolation of indigo-red.
Dawbarn's red-ring reaction and significance of it.
Clinical tests for phenol, catechol and hydrochinon.
Isolation for medico-legal purposes of catechol and hydrochinon.
Clinical test for neutral (unoxidized) sulphur, cystine, etc.

THE COMPOUNDS OF SULPHUR.

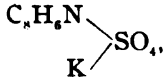
The sulphur compounds are (1) oxidized (sulphates) and (2) unoxidized. Three kinds of sulphur compounds occur in the urine: the preformed sulphates, the ethereal sulphates, and the unoxidized sulphur. The two forms of sulphates constitute the oxidized sulphur.

Chemistry.—The *preformed* sulphates, $Na_2SO_4 10H_2O$, and K_2SO_4, are known also as the *mineral* or *neutral* sulphates, and are compounds of sulphuric acid with sodium and potassium. They are readily soluble in water, hence occur in solution in the urine, but not in sediments nor in calculi. The rarer constituent, calcium sulphate, $CaSO_4$, is occasionally seen in sediments. (See "Sediments.")

The sulphuric acid derived from the metabolism of proteins combines not only with the circulating alkalies (Na,K) in the blood, but also with circulating aromatic substances (indoxyl, phenyl, cresyl, etc.); hence we have *ethereal sulphates,* called also *aromatic* or *conjugate* sulphates,—potassium indoxyl sulphate ($C_8H_8NKSO_4$), potassium skatoxyl sulphate ($C_9H_8NKSO_4$), phenyl potassium sulphate ($C_6H_5KSO_4$), cresyl potassium sulphate ($C_7H_7KSO_4$). Hydrochinon (hydroquinone) $C_6H_4(OH)_2$ (1,4), and catechol (pyrocatechin, brenzcatechin) $C_6H_4(OH)_2$(1, 2) also occur as potassium salts combined with sulphuric acid. The aromatic sulphates as a rule occur in solution; indoxyl oxidized to indigo-blue occasionally occurs in sediments and in calculi.

Chemistry of Indoxyl (Indican).—During putrefaction in the intestine indole, C_8H_7N, is formed, which in the blood is oxidized, becoming indoxyl, C_8H_6NOH. Indoxyl unites with sulphuric acid, forming indoxyl sulphonate and water, $C_8H_6NO.HSO_3 + H_2O$. Indoxyl sulphonate reacts with potassium hydrophosphate in the urine, forming indoxyl-potassium sulphonate and potassium dihydrophosphate, *i. e.*, $C_8H_6NO.KSO_3 + NaH_2PO_4$.

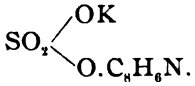
Graphically the formula for indoxyl is usually represented as



the substitution of the hydrogen in sulphuric acid being illustrated as follows:



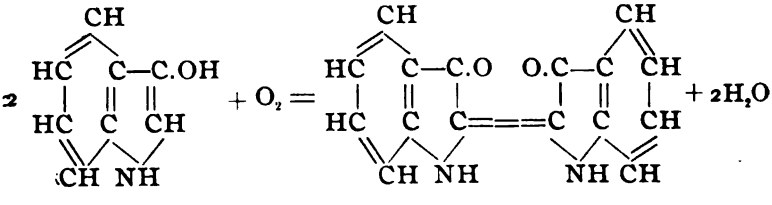
If R is understood to represent any aromatic radical, then substituting C_8H_6N for R, and K for H, we have



Indoxyl occurs in the urine in solution and as such is not precipitated. Oxidation converts it into indigo-blue, according to the equation

$$\underset{\text{Indoxyl-potassium sulphonate (sulphate)}}{2C_8H_6NKSO_4} + O_2 = \underset{\text{Indigo-blue}}{2C_8H_5NO} + \underset{\text{Potassium Acid sulphate.}}{2KHSO_4}$$

Graphically the change of indoxyl into indigo-blue may be represented as follows, disregarding the element of potassium:



Two molecules of indoxyl plus two atoms of oxygen equal indigo-blue plus two molecules of water.

Chemistry of Skatoxyl.—The substance $C_9H_8NOSO_2OH$ occurs in less amount in the urine and its presence is disputed. It is formed from skatole in the organism, just as indoxyl is from indole, but the reasons for the transformation of the major portion of the tryptophane group of the protein molecule into indole are not well understood (Hawk).

Chemistry of Indigo-Red.—The substance is isomeric with indigo-blue and is always formed along with it in the tests. It may also occur already formed in concretions and in some cases in the freshly voided urine, as in pyelocystitis.

Chemistry of the Phenyl and Cresyl Compounds.—These occur in the urine as phenyl and cresyl potassium sulphates respectively, $\left.\begin{matrix}C_6H_5O\\KO\end{matrix}\right\}SO_2$ and $CH_3.\left.\begin{matrix}C_6H_4O\\KO\end{matrix}\right\}SO_2$ respectively.

Phenyl sulphate is supposed to be formed intermediate—as to place in the intestine—between indoxyl and skatoxyl.

Chemistry of Hydrochinone and Catechol.—These substances have the same formula, $C_6H_4(OH)_2$, but differ in the molecular arrangement of the hydroxyl group, hydrochinone substituting OH for H_1 and H_4 and catechol for H_1 and H_4. Both are excreted as conjugate sulphates, and urine containing these bodies darkens on standing. Both occur as a result of the ingestion of carbolic acid, but catechol is said to have its source also in the decomposition of vegetable food.

The term "acid sulphates" is used to denote the oxidized sulphur, *i. e.*, the sum of the preformed and conjugate sulphates.

The unoxidized sulphur, also called *suboxidized* or *neutral*, is made up of taurocarbaminic acid, cystine, cysteine, thiosulphates, sulphocyanides, oxy-, alloxy-, and antoxy-proteic acids, uroproteic, and uroferric acids.

Cystine is found in urinary sediments and calculi; the other neutral sulphur compounds appear dissolved in the urine. The neutral sulphur compounds consist of two classes, the readily oxidizable and the difficultly oxidizable or "salivary" and "biliary" compounds.

Taurocarbaminic acid, $C_3H_8N_2SO_4$ is an uramic acid, a compound of taurin, $C_2H_7NSO_3$, and carbamide, CONH, the synthesis probably being effected in the kidneys by the union of taurin with urea, forming ammonium taurocarbaminate.

Cystine is denoted by the formula $[SC(CH_3)NH_2CO_2H]_2$ a derivative of alpha-amidopropionic acid, and is represented as follows:

HOOC. $CHNH_2$.CH_2S. SCH.$CHNH_2$.COOH,

that is, alpha-diamino-beta-dithio-dilactic acid.

It is a constant product of the decomposition of albumins, but normally is not present in urine, hence resembling alkapton in that its presence indicates an unknown metabolic anomaly, less than 150 cases having been reported. Diaminuria is likely to occur in the same individual (see "Ptomaines").

The presence of it in the urine favors the formation of cystin calculi.

Cysteine.—The urine contains, in all probability, a neutral sulphur compound related to cystine, presumably intermediary between the proteins and the highly oxidized sulphates split off from them. On feeding dogs with halogen benzoles peculiar products appear in the urine, termed mercapturic acids, containing both sulphur and nitrogen and apparently united to glycuronic acid. Baumann and Preusse succeeded in isolating a bromphenyl-cysteine in this way, hence the assumption that a cysteine complex is normally produced in the sulphur metabolism of the dog.

Sulphocyanates.—The salivary glands excrete potassium sulphocyanate, KCNS, which is swallowed, reabsorbed from the bowel, and excreted in the urine.

Thiosulphates.—Normally compounds of thiosulphuric acid, $H_2S_2O_3$, occur only in traces (10 milligrammes per liter) or are absent altogether.

Physiology.—The oxidized sulphur (sulphuric acid) is derived mostly from the metabolism of proteins, and the excretion of it is more or less parallel with that of the nitrogen. The *preformed sulphates* result from combination with circulating alkalies. The *aromatic sulphates* result from bacterial putrefac-

tion of proteins in the intestine, and of the nucleo-albumins, pancreatic and intestinal juices, bile, and intestinal mucus. According to older authorities, the quantity of the ethereal (aromatic) sulphates is directly proportional to the intensity of the putrefactive processes in the intestine. As a result of recent experiments Folin differs from this opinion, except with reference to indoxyl. He claims that the ethereal sulphates represent a diet containing little or no protein, but indoxyl alone may be taken as a rough index of the extent of the putrefactive processes.

Physiology of Indican.—Proteins reduced to tryptophane by the pancreatic juices or the action of anerobic bacteria by bacterial decomposition yield indole, especially from the action of the bacillus coli. Indole is normally absorbed in the cæcum and colon, and most of it is used by the tissues, only 30-60 per cent of it reaching the liver, where it is converted into indoxyl. Normal urine contains from 5 to 25 milligrammes per 24 hours in the case of adults on a mixed diet.

Physiology of Skatoxyl.—This substance is supposed to be formed in the lower part of the intestinal tract, while indoxyl is formed higher up. According to Combe the normal quantity is from 0.005 to 0.010 per liter, and from 0.010 to 0.15 per 24 hours.

Physiology of Phenyl and Cresyl Compounds.—The source of these substances is much the same as that of indoxyl, and they are increased by the same circumstances; but when the phenyl compounds are increased indoxyl is not necessarily increased. They are supposed to be formed in an intermediate place in the intestines between indole and skatole. The total amount of the two per 24 hours in the urine ranges from 17 to 51 milligrammes, that of cresyl exceeding the phenyl.

Physiology of the Unoxidized Sulphur.—This portion of the sulphur which has escaped oxidation,—constituting from 12 to 25 per cent. of the total sulphur,—is derived mostly from taurocholic acid of the bile, in less amount from the sulphocyanide of the saliva, and in fever urine from thiosulphates. The salivary sulphur is readily oxidized, the biliary difficultly so.

Total Quantity of Sulphur Compounds.—The *quantity* of the total sulphuric acid reckoned as SO_3 ranges from 1.5 to 3 grammes per 24 hours, of which the ethereal and the neutral are each about one-tenth.

The amount of *sulphuric acid* united to form the *preformed sulphates* is about 2 grammes in 24 hours and the amount of these sulphates, from 3 to 4 grammes in 24 hours. Of the preformed sulphates the sodium salt is in excess over the potassium. The ratio of the total SO_3 to nitrogen averages about 1 to 5 and to urea about 1 to 12.

Physiological Variations in Quantity.—The *preformed sulphates* are *increased* by foods or drinks rich in sulphates or oxidizable sulphur compounds, by meat diet, active exercise, and oxygen inhalations. They are *decreased* by fasting, vegetable diet and use of carbolic acid or phenyl-containing compounds.

The *ethereal sulphates* are *increased* fourfold by meat diet, by gormandizing and by bolting of the food; they are *absent* in the urine of the new-born, and from the first to fifth year are excreted in quantity only from 0.05 to 0.08 gramme (adults 0.1 to 0.15). They are decreased by vegetarian diet, farinaceous food, milk diet, sour milk, kumyss, (Kephir, Kazol), and by adding 100 grammes of lactose to the diet; by light eating and thorough mastication. Infants void less on mother's milk than on cow's.

The quantity of ethereal sulphates varies at *different times* of the *day*, according to Poehl averaging 0.061 grammes per liter at 8 A. M., 0.027 at noon and 0.147 at 8 P. M.

Considering the *phenol* excretion separately we find that the cresyl compounds are *increased* by a vegetable diet and by the administration of benzole, tyrosine, carbolic acid, lysol, salol, etc.

The *neutral sulphur* varies according to the constituents: *taurocarbaminic acid* is derived from the taurocholic acid of the bile, and is increased by the ingestion of taurin.

Cystine, when it occurs, varies from traces up to 1 gramme or more per 24 hours, and the neutral sulphur is correspondingly increased until it becomes 60 per cent. of the total sulphur.

Sulphocyanate of potassium occurs in quantity 0.035 gramme per liter, about one-third the neutral sulphur, and is greatly increased by inhalation of carbon disulphide.

Cystine occurs normally in small quantities, 0.015 gramme per 24 hours.

Pathological Variations in Quantity.—The *preformed sulphates* are *increased* in acute febrile and inflammatory diseases: pneumonia, meningitis, encephalitis, myelitis; in delirium and in rheumatism; in leukemia, some eczemas, and in *diabetes* (mellitus and insipidus).

The preformed sulphates are *decreased* in poisoning by carbolic acid, etc., in convalescence from acute diseases when appetite and metabolism are diminished, and in chronic diseases, especially of the skin and kidneys.

The ethereal sulphates are *increased* by circumstances favoring protein putrefaction: morbid conditions; putrid food; eggs containing hydrogen sulphide; morbid conditions of the teeth and mouth infecting the food; insufficient mastication; any cause of atony or paralysis of the gastro-intestinal tract hindering the progress of the food; *constipation; bowel obstructions; bowel diseases,* and toxemias; absorption of *putrid pus* anywhere in the body; ingestion of *certain drugs;* astringents, alkalies, carbolic acid, etc., turpentine, creosote, oil of bitter almonds, nux vomica; lead poisoning. The phenol compounds are increased in phosphorus poisoning.

Clinical Notes.—Leaving out *pus absorption,* which even in small quantity may increase the ethereal sulphates considerably, we find *intestinal obstruction* to cause the greatest increase, as much as 0.935 gramme having been found by Combe in *cæcal invagination.* In *peritonitis* and *perityphlitis* there is a marked increase. In *constipation* there is a great increase whenever there is atony with stagnation in the upper portions of the intestine. In **enteritis** the amount decreases during periods of diarrhea, but increases during the constipation, as high as 0.627 gramme having been found by Bartoschevitsch. In obstruction of the flow of bile into the intestine, as in *icterus,* the ethereal sulphates are quadrupled in amount.

In all *gastric conditions with hyperacidity* there is considerable increase.

In **akinetic mental conditions** without toxemia an increase is claimed: also in epilepsy after the attacks.

In nephritis, Addison's disease, neurasthenia, and chronic intestinal indigestion an increase may be noted; also in conditions vaguely called lithemia.

The ethereal sulphates are *decreased* by conditions causing absence of trypsin from the small intestines; closure of the pancreatic duct; in acute intestinal catarrh; during periods of diarrhea; and in non-obstructive jaundice; by ingestion of certain drugs: alkaline sulphates, sulpho-carbolates, salicylates, calomel, bismuth; by purgatives, especially castor-oil, mineral waters containing sulphates (Carlsbad, Marienbad, Hunyadi Janos, Pluto).

Clinical Notes on Indicanuria.—On account of the close relation of indicanuria and albuminuria it is said that some of the insurance companies are beginning to regard the former with disfavor as an early sign of nephritis. The author has occasionally found it conjoined with a trace of albumin and a few hyaline casts in persons showing no other evidence of nephritis. Occasional increase of indican is so commonly found by the author that from a life insurance viewpoint only persistence of it becomes of interest.

☞ Dr. F. C. Askenstedt, of Louisville, says: "Clinical experience suggests that the bacteria usually found in the intestinal canal, when permitted an unusual growth, may lead to a gradual intoxication. Inasmuch as urinary indican is always derived from bacterial growth and does not serve any useful purpose in the economy of man, its presence can not be considered physiological. Continued high excretion points unerringly to a seriously impaired digestive function."

In typhoid fever it has been claimed that marked indican reactions are not obtained before the 3d or 4th week, while in simple fevers the reaction is noticed at the very beginning.

Concetti states that indican is increased in the urine of children suffering from tuberculosis, anemia, and scrofulosis of the lymph glands.

☞ Askenstedt has noticed a great excess of indican in cancer of the stomach and liver, in functional motor insufficiency of the stomach, in hyperchlorhydria, neurasthenia, dysentery, chronic diarrhea and pulmonary tuberculosis in the second stage.

It is probable that the safest diagnostic guide in cases of suspected gastro-intestinal toxemia is the coefficient of Combe, expressing the ratio of the sulphuric acid of the ethereal sulphates to the total nitrogen. (See "Ratios and Coefficients.")

It must not be forgotten, however, that indican in the urine is found abundantly during the season when strawberries, rhubarb, and asparagus are abundant. During the latter part of May and in June, in Chicago, brilliant indican reactions are extremely common, so much so as to suggest relationship to the diet of the people at this season. It has been suggested that the reaction in some cases may be due also to the eating of eggs which have been kept in storage.

Diagnostic Points.—When indican is abundantly present in the urine its source may usually be ascertained by the following: if the patient is a hearty eater, especially of meats, regulation of the diet will negative the indican reaction; cases in which it is due to cachexia are recognized by the uniformity with which the reaction occurs throughout the day and the fact that administration of bismuth does not diminish it, but milk and alkaline waters cause diminution. If the indicanuria originates from foci of pus, the reaction is brilliant, the amount of indigo-blue is large, and the greatest amount is found at night. Meals have no effect upon pyogenic indicanuria, nor do bismuth, milk or alkaline waters affect it. If the indican is due to intestinal putrefaction it will be diminished by a milk diet and by bismuth salts, but alkaline waters will increase it. The amount of indican is moderate, reaching its maximum five hours after meals, in cases due to intestinal indigestion.

A brilliant indican reaction in urine of specific gravity below 1020 will usually show a pathological condition, which can be determined as above.

In cases of intestinal autointoxication the sinusoidal electric current may be employed for the diagnosis as follows:

The current is applied to the abdomen by means of large electrodes in a broad belt. Both electrodes of a strong current are applied to the abdomen for about 15 minutes. In cases of autointoxication the indican is greatly increased after the application

of the current, but with subsequent treatment on successive days the amount becomes less and less. The same is true of the total ethereal sulphates.

Pathological Variation in Quantity of Neutral Sulphur.—The *neutral sulphur* varies pathologically according to different circumstances; *taurocarbaminic acid* is *increased* by biliary obstruction, as in obstructive jaundice, or after ligation of the common duct in dogs. **Cystine** in the urine constitutes a congenital anomaly which runs in families, and except for the tendency to form calculus is not inconsistent with the general health. An increase in cystine increases the neutral sulphur considerably.

Thiosulphates are found in the urine of typhoid fever patients.

Clinical Tests for Sulphur Compounds.—Make a solution of barium chloride containing a little hydrochloric acid as follows: pure barium chloride, 40 grammes, dissolved in 160 c.c. of distilled water, to which 8 c.c. of pure concentrated hydrochloric acid have been added.

To 10 c.c. of the urine add 3 c.c. of the above; mix well, and observe the resulting opacity due to precipitation of the preformed sulphates as barium sulphate; if the turbidity is *milky* the preformed sulphates are present in normal amount; if *creamy* they are excessive; *if less than milky,* diminished. Normally the precipitate is at first voluminous, but, being heavy, settles and fills about one-half the concave bottom portion of the test-tube in the course of 18-24 hours.

Clinical Tests for Ethereal Sulphates as a Whole.—To test for the ethereal sulphates proceed as follows: 25 c.c. of urine are treated with about the same volume of an alkaline barium chloride mixture (2 volumes of a solution of barium hydrate and 1 volume of a solution of barium chloride, both saturated at ordinary temperatures) and filtered after a few minutes, the mineral (preformed) sulphates as well as the phosphates being thus removed. The filtrate is then strongly acidified with hydrochloric acid and boiled, when the occurrence of a precipitate will be referable to ethereal sulphates. Or, after 24 hours' standing, filter the urine obtained in testing for preformed sulphates, and acidify with hydrochloric acid; add 1 c.c. of the acid to 10 c.c. of

the filtered urine, and boil. The ethereal sulphates are split and appear as barium sulphate, which is precipitated.

Clinical Tests for Indican.—The principle of all the tests is to oxidize the colorless indoxyl into indigo-blue and extract the latter with chloroform. This can be done in two ways; first, that of Jaffé and Stokvis, who use hydrochloric acid, oxidizing agent, and chloroform; and, second, that of Obermayer, who uses a weak solution of ferric chloride in hydrochloric acid and further adds chloroform without other oxidizing agent.

1. **Jaffé's Test.**—To 10 c.c. of urine add a like quantity of concentrated hydrochloric acid; mix by inverting; warm to blood heat; add 1 drop or at most 2 drops of an oxidizing agent, as, for example, a saturated solution of the ordinary household disinfectant, "chloride of lime;" and from 2-3 c.c. of chloroform and shake well. As the chloroform sinks to the bottom a blue color in it signifies presence of indican.

☞ 2. **Obermayer's Test.**—Make up a solution of ferric chloride, Fe_2Cl_6, 2 to 4 grammes in 1000 c.c. of concentrated hydrochloric acid. The urine is first treated with 20 per cent. solution of plumbic acetate, filtered, and to the filtrate are added equal parts of the test liquid made as above. In a few minutes the blue color appears.

Potassium iodide gives a violet color and thymol a bluish-green. The test may be modified, adding a little chloroform, corking the tube and inverting several times without shaking. The chloroform becomes blue in proportion to the indican present, increasing on standing.

Another method of using the test is to shake the filtrate from the lead acetate vigorously with the reagent liquid, and to shake again after adding chloroform.

☞ 3. **The Author's Method.**—A clear solution of neutral lead acetate is made by dissolving 20 grammes of the pure crystals of bright glassy appearance (not a dull white powder) in 80 c.c. of distilled water, which is then boiled to drive off any CO_2 and cooled before it is used.

According to the specific gravity of the urine and to other considerations there are added to 10 c.c. of it from 1 to 25 drops of

the lead acetate solution. A white precipitate at once is seen. The mixture is well shaken and filtered through two thicknesses of filter paper or until it comes through clear. All containers must be scrupulously clean.

The considerations which affect the amount of lead acetate to be used are the following: urines of low specific gravity (below 1015) require usually but a few drops, one to five; but if sugar be present a large number of drops, 15 to 25, must usually be used. The same is true of concentrated urines of high specific gravity (1030 or upwards), or (1) any urine with a sediment of urates, or (2) an ammoniacal urine with abundant phosphatic sediment, or (3) a urine highly colored from any cause. If bile is present, several c.c. of the lead solution may be required. If albumin is present the urine must be boiled (with slight acidulation if necessary) and filtered.

In the case of urines free from the above mentioned conditions the routine use of ten drops of lead solution may be practiced. But if no blue color is obtained, the operator must vary the amount of drops of lead used until at least some blue is obtained and then make further test with a little more and a little less lead until the maximum color is obtained.

After the urine has been filtered clear from the precipitate made by adding the lead, warm it under the hot water hydrant, or in the hot water bath, until the tube is hot to the hand. (The best temperature has been determined by Askenstedt to be from 45° to 60° C., after the acid has been added as below.) When it is thus warm, then add an equal volume of Baker and Adamson's c. p. hydrochloric acid, of specific gravity, 1.19. Mix well, then add just one drop of the oxidizer, a saturated filtered solution of "chloride of lime" (bleaching powder, disinfecting powder), sold in small metallic boxes as a household disinfectant. This oxidizer is kept in an uncorked bottle, exactly contrary to the advice of the various authorities. The reason why it should be kept in an uncorked bottle is in order that the free chlorine in it may have an abundant opportunity to escape. It is the presence of too much of this free chlorine which bleaches the blue color we desire to obtain.

After the drop of oxidizer has been cautiously added, do not shake, but mix gently by inverting the tube once or twice. If now the mixture is seen to darken, the presence of indican is shown. In any event further add 2 c.c. of chloroform, mix gently at first, inverting the tube several times, then begin to shake, and finally shake vigorously. If indigo-blue has been obtained by oxidation of the indoxyl, the chloroform settling down to the bottom of the tube will be **blue** in color. The chloroform-indigo mixture must be clear. If it is cloudy, the amount of lead acetate originally used is insufficient. If it is colorless, too much lead has been used.

For quantitative purposes it is usually sufficient to observe the depth of color of the chloroform, light blue being normal, deep blue denoting excess; but care must be taken to be sure that all the indigo has been extracted by shaking. This may be done by allowing the mixture to settle, decanting off the supernatant urine, adding more chloroform and shaking again. If there is still indigo left, the fresh supply of chloroform will again be colored blue. This means that the amount of indigo formed was too much for the 2 c.c. of chloroform originally used to extract, or that the operator failed to shake with sufficient vigor and for a long enough time.

This method of procedure is, on the whole, better than the tests described by the various learned authorities who lay much stress upon things immaterial to the test and overlook the two great issues, namely, (1) interfering bodies, and (2) temperament of best oxidation.

The test for indican is clouded by these two perplexities. The author's method is often unsatisfactory, very, but in the long run is superior to the ones described in the books, hence is desirable for routine clinical purposes.

Clinical Notes.—When the urine is of specific gravity 1015 or lower, a brilliant blue with the author's indican test and a marked violet with the author's modification of Goldschmiedt's glycuronate test, strongly suggests the presence of gastrointestinal toxemia. In urines of high specific gravity it is likely that the indigo blue will be abundant from mere concentration of the normal amount of indoxyl, hence the test is not so significant.

Laboratory Note.—In case the urine darkens with hydrochloric acid, with a blue tint by reflected light, but the test with oxidizer and chloroform shows no blue, omit one or the other or both of these last two substances. In a few cases the hydrochloric acid itself appears to oxidize sufficiently well, and further addition of oxidizer destroys the color. Occasionally the chloroform appears to destroy the color. The author advises the use of the purest chloroform obtainable.

The test described above is really Jaffé's, the technique alone being that of the author. If the test fail, repeat at once, using the same test tubes, washed clean with hot water.

4. Jaffé's Test Modified by Daland.—The emulsion with chloroform can also be prevented by doing away with shaking, as in the following test of Daland, which gives excellent results in some samples of urine, and should certainly be tried when other tests fail: to 1 c.c. of filtered urine add one drop of a 1 per cent. solution of potassium chlorate, 5 c.c. of chloroform and 10 c.c. of concentrated hydrochloric acid of specific gravity 1.19. Mix by pouring slowly and repeatedly from one tube to another and the chloroform is colored blue if indican is present in notable amount. Not only the chloroform but the whole liquid may become intensely colored (dark blue) when indican is abundant.

The test may show much more color when the tube is allowed to stand several hours than it does at first, if indican is not in great excess.

Other Oxidizing Agents.—Hydrogen peroxide, potassium permanganate, copper sulphate, osmic acid, sodium persulphate, ammonium persulphate and potassium chlorate, respectively, have been advocated as oxidizing agents by different analysts, which is practically a tacit recognition of the difficulties attending the test. Gürber advises that to one volume of urine two volumes of hydrochloric acid be added; from 2-3 drops of a 1 per cent. solution of osmic acid, added sometimes before, sometimes after the acid; and 2-3 c.c. of chloroform, with shaking. Highly colored urines are to be decolorized with basic lead acetate solution (using one-eighth volume and filtration). Porter uses as an oxidizing agent a 0.5 per cent. solution of potassium permanganate, and

publishes a table of color reactions which may be seen in Saxe's "Examination of the Urine." In the writer's experience the particular oxidizing agent employed has very little to do with the success of the test, almost any one serving the purpose well enough, the fundamental trouble being caused apparently by the temperature, the hydrochloric acid, and the interfering substances in the urine. Rossi advises 1 drop of a 10 per cent. solution of ammonium persulphate for an oxidizing agent, together with hydrochloric acid, urine and chloroform as usual, but this does not help matters, as explained above. Lavelle adds 2 or 3 c.c. of Obermayer's reagent to 10 c.c. of urine and 2 or 3 c.c. of concentrated sulphuric acid, keeping the tube in cold water while mixing; 2 or 3 c.c. of chloroform are further added, with vigorous shaking. This test also does not remove interfering substances. Amann uses sodium persulphate as an oxidizing agent, and shakes with benzole instead of chloroform, a blue color showing indican, a violet indoxyl and skatoxyl or indigo-red, and a rose indigo-red mixed with skatoxyl. This process, however, does not remove the emulsioned substances.

☞ **Quantitative Determination of Indoxyl.**—The best method is that of Askenstedt, of Louisville, Kentucky, as follows: Add to some urine one per cent. by weight of mercuric chloride in substance. Wait until it is dissolved, then filter. To 10 c.c. of the filtrate warmed to blood heat or slightly over add 10 c.c. of a 0.3 per cent. solution of ferric chloride, Fe_2Cl_6, in hydrochloric acid, sp. gr. 1.19, and mix by inverting the tube once, then add quickly 8 c.c. of chloroform, and extract the nascent indigo- blue by shaking the tube three minutes, holding it in a horizontal position. After this let the chloroform fall to the bottom of the tube; then pour off most of the supernatant fluid; fill the tube nearly full with water; invert it a few times to wash the chloroform and let this again precipitate in the tube; pour off most of the water. Repeat twice this process of washing, taking care that no chloroform escapes with the wash water, and allowing not more than 2 or 3 c.c. of the last wash water to remain over the chloroform. Now add from 13 to 15 c.c. of alcohol and mix by inverting. A clear blue fluid should result. If hazy, add one or two c.c. more

of alcohol until the fluid clears up. Compare the color of this fluid with an equal quantity of a standard solution of indigo-blue in the second test-tube by holding the two test-tubes in front of a white surface. The standard solution is made by pouring into the empty second tube a quantity of water equal to the amount of the fluid in the first tube, then dropping the stock solution of indigo-blue into the water (the drop of the stock solution in forming at the opening on the pipette should hang from the rim of the opening only, and not be augmented by a flow from the *outside* of the pipette), inverting the tube after each drop, until both solutions have the same amount of blue color. If this requires four drops of the stock solution the percentage is 0.0004; if five, 0.0005; if six, 0.0006, etc.

The indican extract will usually prove to be slightly greenish. By adding one or more drops of the picric acid solution to the standard solution in the test-tube, this can be made to conform to the color of the extract. Urine containing 0.002 per cent. or more of indican, or giving a blackish extract, should be diluted with equal quantity of water and retested.

By the use of Askenstedt's quantitative method the ratio of indican to urea may be calculated thus: urea, 3 per cent.; indican, 0.00072 per cent.; ratio of indican to urea 1 to 4166. This ratio Askenstedt deems a better basis for a standard of the decomposition of proteins than the percentage or total amount of indican. A ratio of indican to urea greater than 1 to 1500 is normal; one of 1 to 1500-1000 shows slight excess, 1 to 1000-500 moderate excess, and any ratio below 1 to 500 a great excess. Askenstedt advises an adjustment of the temperature of the urine and the acid such that when mixed a temperature between 45° and 60° C. is obtained.

Laboratory Notes on Askenstedt's Method.—The picric acid solution used is in strength one part by weight to about 5000 of alcohol. The indigo solution recommended is made from indigotine of commerce dissolved in sulphuric acid and used with a certain medicine dropper, each drop delivered containing exactly 0.00615 milligrammes of the indigotine. In order to make this solution, proceed as follows: find out how many drops of

strong sulphuric acid a given medicine dropper will deliver from 1 c.c. Multiply this number by 0.00615 to obtain the amount of indigotine which should be dissolved in each c.c. of the sulphuric acid used. Keep the indigotine solution in an amber bottle.

Denatured alcohol may be used in the performance of the test for dissolving the indigo blue formed in the urine, and if one part of mercuric chloride be dissolved in 1000 of the alcohol thus used, fading of the color is prevented when the tube is allowed to stand.

2. Ellinger's Method.—If the urine is neutral or alkaline make it feebly acid with acetic acid and to 50 c.c. in a small beaker or casserole add 5 c.c. of basic lead acetate solution (strength not stated, as usual). Mix thoroughly and filter. Transfer 40 c.c. of the filtrate to a separatory funnel (Fig. 14); add an equal volume of Obermayer's reagent, and shake with 20 c.c. chloroform. Pipette off the supernatant urine from the chloroform after settling and shake again, repeating the extraction until the chloroform is colorless. Unite all the chloroform extracts; filter through a dry filter into a dry flask. Distil off the chloroform; heat the residue on the boiling water bath for 5 minutes in the open flask, and wash the dried residue with hot water, washing until the water is no longer colored, filtering the wash water if particles of indigo-blue separate, extracting the indigo from the residue with chloroform again, and filtering again through a dry filter.

To the washed residue add 10 c.c. of concentrated sulphuric acid; heat on the water-bath for 5-10 minutes; dilute with 100 c.c. of water, and titrate the blue solution with a solution of potassium permanganate originally containing 3 grammes per liter, but diluted for this purpose with 40 volumes of water. The end-reaction is shown by the complete disappearance of all blue color and the formation of a pale yellow.

In criticism of the above the author has only to repeat his statement that in some cases the chloroform fails to extract the indigo-blue, hence the first requisite is to determine whether or not this can be done by quantitative testing.

3. Strauss' Quantitative Indican Method.—To 20 c.c. of urine

add 5 c.c. of 20 per cent. lead acetate and filter. To 10 c.c. of filtrate corresponding to 8 c.c. of urine in a separatory funnel add 10 c.c. of Obermayer's reagent and 5 c.c. of chloroform. Cork and shake gently. In two minutes shake again and let out chloroform. Add 5 c.c. more of chloroform, shake, and so until no more blue is extracted. Compare 2 c.c. of the united extracts in a small test-tube with a solution made by dissolving 1 milligramme of Kahlbaum's purest indigotine in 1000 c.c. of chloroform and kept in the dark. Add chloroform drop by drop until the color is exactly the same in both tubes, which should be small and of exactly the same size. Let *a* equal the total chloroform

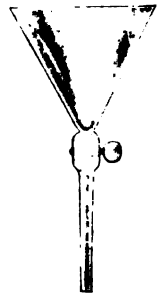


FIG. 14.—Separatory Funnel.

used for extraction, and *x* the chloroform used for dilution, and the latter can be calculated from the formula *a* times $\frac{5}{2}$. In normal urine from 5 to 10 c.c. of chloroform will extract all the indican from 8 c.c. urine.

In criticism of the above the author has only to refer to difficulties already described. The urine mixture should certainly be warmed as recommended by Askenstedt.

☞ **Indican Reaction in Presence of Iodides.**—When the patient is taking iodides the chloroform extract is likely to be colored a bright pink or red. This color can be removed by addition of a little of a strong solution of sodium thiosulphate (hyposulphite).

Let the chloroform extract settle, decant the supernatant acid mixture, replace with a small quantity of strong solution of sodium "hyposulphite" and shake again. Or shake the decanted chloroform extract with dilute solution of potassium hydroxide.

Errors From Codeine and Formaldehyde.—Codeine in the urine may give a deceptive blue with the Obermayer test

Presence of formaldehyde prevents formation of the blue when the indican test is tried. Askenstedt claims that even the vapor of it in the room may prevent the reaction.

Clinical Tests for Skatoxyl and Indigo-Red.—In Jaffé's test for indican the amount of skatoxyl may be inferred by the color above the blue. Skatoxyl carbonate yields a violet color and the sulphate a red, but indigo-red is held by some authorities to be responsible for these same colors. The color may appear in the chloroform extract.

Indigo-red is best differentiated from skatoxyl and indican by *Rosenbach's reaction:* to the boiling urine is added concentrated nitric acid, drop by drop, which gives a Burgundy-red color if indigo-red (urorubin, urorhodin) is present in abundance. Held to the light the color may assume a bluish tinge, and the foam a blue color. In some cases addition of from 10-20 drops of acid will suddenly change the color from red to yellow, while in others the red is not changed.

Differentiation of Iodine, Indican and Skatol in Jaffé's Indican Reaction.—According to B. Spiethoff if the urine contains iodine the filter paper will turn blue all over when the urine to which has been added some chloroform is being filtered, while the red chloroform will remain at the bottom of the funnel. The blue color of the filter paper fades gradually. The color of the sediment will also vanish, but it will last in the presence of skatol or indican.

Clinical Notes.—Some writers attribute Rosenbach's reaction to skatol excreted as skatol-carbonic acid and found in tubercular ulceration of the intestines, gastric or intestinal carcinoma or pneumonia. If the Rosenbach reaction is constant and continued in spite of medical or surgical measures it is of bad prognostic significance.

Isolation of Indigo-red.—Inasmuch as indigo-red may be present in large amounts in urines which do not yield Rosenbach's reaction, its presence should be sought for as follows: (1) On

addition of nitric acid in the cold, the urine turns red; (2) Jaffé's test for indican gives a red color, especially if the mixture is heated; allow the urine to cool, neutralize the acid with sodium hydroxide and shake out with ether, when the latter will take on a fine red color and give the absorption spectrum characteristic of indigo-red. Crystals of it may be obtained by evaporating the ethereal extract, which yields dark reddish brown or chocolate brown needles or plates *insoluble* in water, dilute acids, or alkalies, but *soluble* in alcohol, ether, chloroform and glacial acetic acid (readily in the last). Its solutions are cherry-red. The crystals sublime with the violet-red fumes at 295° to 310° (563°-590° F.)

The solutions give a characteristic absorption spectrum. If the alcoholic solution is made alkaline with sodium carbonate, a little glucose added, and the mixture warmed gently, reduction takes place and the red color disappears, but on shaking in the air the color returns.

The Red-ring Reaction.—In testing urine for albumin with concentrated nitric acid by the contact method (floating the urine upon the acid) a color ring is always present, seen best by holding the tube against white paper. This ring varies normally in color from light-yellow, faint greenish-yellow and yellow to dull brown, according to the specific gravity of the urine. Occasionally, however, a *bright red ring* appears, usually in the case of those who are habitually constipated. This red should not be confounded with the dull brown so commonly seen with nitric acid in any urine of high specific gravity. Occurrence of the reaction is not common, but when present it is significant of marked intestinal toxemia. Dawbarn, of New York, applies the term "walking cesspool" to patients in whom the reaction occurs, but is unable to identify the chromogen which causes the color to appear.

Clinical Tests for Phenol.—The simplest clinical test is to boil the urine with the nitric acid, noting the odor which, if suggesting oil of bitter almonds, shows presence of phenol; let cool and add bromine water, which gives a yellow precipitate (tri-nitro-bromphenol). To another portion after cooling add sodium hydroxide in excess, and if phenol is present an orange-red color

will be obtained. Albumin must be removed before this test is applied.

Clinical Tests for Catechol and Hydrochinone.—To show presence of *catechol* add a little sulphuric acid to the urine, boil, cool and extract with ether. Evaporate the ether, dissolve the residue in a little water, and apply the following tests: add dilute ferric chloride solution,—a green color is evolved. Add a little tartaric acid and then ammonia,—the green color changes to violet, but on acidifying with acetic acid the green color reappears.

Alkalies cause the color of a solution of pyrocatechin (catechol) to darken, and alkaline cupric solutions are reduced by it.

Hydrochinone (hydroquinone) is recognized by boiling the urine with a dilute solution of ferric chloride and obtaining the odor of liberated quinone. Urines supposed to be rich in pyrocatechin are for the most part really cases of alkaptonuria (see "Alkapton"). Pyrocatechin is, however, suggested when there is a history of carbolic acid taking.

Isolation.—Since the isolation of these substances is sometimes of medico-legal importance in cases of suspected carbolic acid poisoning, the urine should in such cases be elaborately analyzed as follows: 1000 c.c. of urine are treated with 70 c.c. of concentrated hydrochloric acid, and distilled until about one-fourth of the total amount has passed over. The ethereal sulphates are thus decomposed, and phenol and cresol are found in the distillate. Their presence can be demonstrated by adding bromine-water when tribromphenol crystallizes out on standing. Hydrochinone and pyrocatechin (catechol) remain in the acid solution. To demonstrate their presence, this is evaporated to about 100 c.c., and on cooling extracted with an equal volume of ether. Hydrochinone and catechol, together with the aromatic oxy-acids, are thus removed. On adding a dilute solution of sodium carbonate to the ethereal solution the aromatic oxy-acids are transformed into the corresponding sodium salts. The ethereal extract, which now contains only the dioxy-benzole, is evaporated to dryness; the residue is dissolved in a little water, and the ferric chloride test applied as above to one portion of it. To the other portion add lead acetate solution and filter. The filtrate contains the

hydroquinone, which can be isolated by acidifying and extracting with ether and evaporating. Dissolve the crystalline residue in a little water and apply the ferric chloride test as above with reference to the penetrating odor of quinone.

Clinical Test for the Neutral Sulphur.—Drop a small piece of zinc into 10 c.c of urine, add enough strong hydrochloric acid to cause evolution of hydrogen gas, and over the mouth of the tube place a piece of filter paper saturated with lead acetate. In a short time the paper becomes black from the formation of hydrogen sulphide, due to combination of the hydrogen with the neutral sulphur of the urine.

Clinical Tests for Individual Neutral Sulphur Compounds.—The *sulphocyanides* in the urine may be detected as follows: precipitate the chlorides in 100 c.c. of urine by adding nitric acid and silver nitrate. The sulphocyanides are also precipitated; filter, wash the residue, shake it up with water and pass hydrogen sulphide into the mixture. Decomposition results and the silver sulphide may be filtered off. The filtrate contains the sulphocyanide and should be distilled. The distillate tested with ferric chloride yields an intense blue (Berlin), insoluble in hydrochloric acid.

Clinical Tests for Cystine.—Urines containing cystine may give off the odor of sulphuretted hydrogen on standing. Acidifying the urine strongly with acetic acid will cause the crystals to form, which are hexagonal and transparent (see "Sediments"). The crystals are soluble in ammonia and reprecipitated by acetic acid. In some cases the crystals occur in the sediment of the urine spontaneously, especially in decomposing urines, and usually in hexagonal plates but sometimes in highly refractive needles with cut ends.

On recrystallization from 10 per cent. ammonia, however, the hexagonal plates appear. Cystine is lævorotatory. It burns on the platinum foil with a bluish-green flame and gives off a peculiar penetrating odor. It is soluble in the alkaline hydroxide solutions and in mineral acids. It is insoluble in water, alcohol, ether and acetic acid, and also in solutions of ammonium carbonate, hence is likely to crystallize out from decomposing urine.

Dissolving the crystals in a dilute alkaline hydroxide solution, and shaking with an ethereal solution of betanaphthalene sulphochloride, give with hydrochloric acid—after the removal of the ether—a precipitate insoluble in cold absolute alcohol. (For quantitative determination see next chapter).

CHAPTER XI.

THE QUANTITATIVE DETERMINATION OF THE SULPHUR COMPOUNDS.

Centrifugal method of Purdy for the preformed sulphates.
Quantitative determinations of the total oxidized sulphur; methods of Folin.
Determination of the ethereal sulphates:—methods of Folin; Swiss method.
Askenstedt's method for indican. (See Chapter XI.)
Ellinger's method for indican. (See Chapter XI.)
Quantitative determination of indigo blue and red by chromometer.
Quantitative determination of phenols: method of Kossler and Penny.
Colorimetric determination of the phenols.
Quantitative determination of the neutral sulphur compounds: Modrakowski's modification.
Osborne-Folin method for total sulphur.
Total sulphur by fusion method with sodium hydroxide, etc.
Separate determination of the two groups of neutral sulphur compounds.
Quantitative determination of cystine (Alberhalden's method).

QUANTITATIVE DETERMINATION OF SULPHUR COMPOUNDS.

Determination of the Preformed Sulphates.—Clinically, the most available method is that of Purdy, with the centrifuge, which merely shows the amount of preformed sulphates, hence is of little diagnostic value.

☞ **Purdy's Centrifugal Method.**—Fill up a Purdy percentage tube to the 10 c.c. mark with filtered urine, from which albumin—if any—has been removed by boiling, cooling, and filtration; add to it 5 c.c. of the barium chloride mixture described above. Mix the contents of the tube well, inverting several times; allow to stand five minutes, then centrifuge at a speed of 1500 revolutions per minute for three minutes and read off the bulk percentage of barium sulphate found. Normally the bulk percentage is less than one, being from one-half to three-fourths of one per cent. by bulk, hence the precipitate in the percentage

tube, if above the first small mark, *i. e.*, one-tenth of a c.c.,—shows a per cent. of sulphate above normal, and if much below half of the space under the first mark, is below normal. One per cent. by bulk was estimated by Purdy to represent 0.25 per cent. of SO_3, or 2.5 grammes per liter, hence ½ per cent. by bulk would represent 0.13 per cent. of SO_3. Multiply by 1.23 to convert to H_2SO_4.

☞ The method is unsatisfactory on account of the small bulk of the precipitate and the tendency of it to settle unevenly.

Clinically, the amount of ethereal sulphates, which is not shown at all by the above process, is of much greater interest.

Quantitative Determination of the Total Oxidized Sulphur (Sulphuric Acid).—Clinically, it is often of importance to make a quantitative analysis of the total sulphur oxidized in the urine, *i. e.*, to obtain the sum of the amount of the sulphuric acid of the preformed and of the ethereal sulphates, as well as the quantity of each of these separately. In addition the amount of unoxidized or neutral sulphur is of interest. The various methods for quantitative analysis are as follows:

☞ **Method of Folin.**—To 50 c.c. of filtered urine are added 5 c.c. of strong hydrochloric acid, and 5 c.c. of a 4 per cent. solution of potassium chlorate. The mixture is boiled,—for the purpose of breaking up the ethereal sulphates,—in a beaker until decolorized. At first the color darkens, but after a few minutes grows lighter, and in five or ten minutes is removed, or, but faint. While still boiling there is slowly added, drop by drop, to the mixture, preferably from a dropper or capillary funnel, 25 c.c. of a ten per cent. solution of barium chloride, the operation taking 5 minutes or more, after which the beaker is transferred to the boiling water bath for half an hour to an hour, until the precipitate of barium sulphate has completely settled, leaving the supernatant fluid clear. It is then filtered through a Schleicher and Schüll filter, No. 589, the weight of whose ash is known, care being taken to keep the paper moist all the time, and to remove the last traces of the barium sulphate from the beaker by adding a little hot water and rinsing with a rod tipped with rubber tubing.

The residue is then washed for half an hour, with hot water and hot 5 per cent. ammonium chloride solution alternately, until the washings give no precipitate with dilute sulphuric acid, not even on standing.

The filter paper is then removed, folded and dried somewhat by gentle pressure between dry filter paper, after which it is transferred to a weighed crucible and covered with 3 to 4 c.c. of alcohol. The latter is ignited and the filter incinerated. A cover is placed on the crucible and the latter heated, at first moderately, but, finally, strongly, with the cover half off, for from five to seven minutes; after which it is removed to a desiccator and cooled over sulphuric acid. (Fig. 15.) After cooling it is weighed, and the difference in weight gives the amount of barium sulphate in 50 c.c. of urine.

Albumin should be previously removed from the urine by boiling, cooling and filtration.

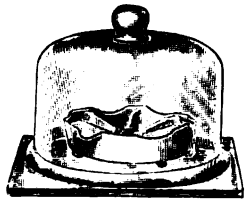


FIG. 15.—Desiccator.

The total SO_3 or H_2SO_4 in the urine is calculated as follows:

100 parts of $BaSO_4$ correspond to 34.29 parts of SO_3, or 1 part of $BaSO_4$ to 0.3429. 100 parts of $BaSO_4$ correspond to 41.99 parts of H_2SO_4 or 1 part to 0.4199.

The preformed sulphates may be determined separately, or, may be obtained by subtraction after determination of the ethereal sulphates.

A platinum crucible is best for the process, though with care a porcelain one may be used. The latter is liable to crack and spoil the experiment.

A weighed Gooch crucible (Fig. 16) may be substituted for the filter paper and platinum crucible, in which case, however,

care should be taken during ignition not to allow the flame to be applied directly to the bottom or sides. Hawk suggests that the Gooch crucible be placed upon the lid of an ordinary platinum crucible supported on a triagle and the flame applied from below the lid used as a bottom to the crucible. Ignition should be complete in ten minutes if organic matter is absent. If the Gooch crucible is used the precipitate should be dried in an airbath, after washing, before ignition.



FIG. 16.—Gooch Crucible.

☞ **Determination of the Ethereal Sulphates.**—The method of Folin is as follows: 200 c.c. of filtered urine are placed in a lipped graduate with a flat bottom, or in a large evaporating dish, and 100 c.c. of a ten per cent. barium chloride solution are added to it at the room temperature. The mixture is well-stirred and allowed to stand 24 hours, in order that the barium sulphate formed by precipitation of the inorganic (preformed) sulphates may settle completely. At the end of that time 150 c.c. of the clear supernatant fluid, corresponding to 100 c.c. of urine, are decanted off and filtered. The filtrate is placed in an Erlenmeyer flask on a wire gauze over the flame; 10 or 15 c.c. of pure concentrated hydrochloric acid and the same amount of a 4 per cent. potassium chlorate solution are added to it, and the whole boiled until decolorized, which requires from five to ten minutes. While still boiling 25 c.c. of a ten per cent. barium chloride solution are slowly added drop by drop. The flask is then placed on the boiling water bath for an hour until the barium sulphate formed by precipitation of the sulphate radical of the ethereal sulphates settles completely. The contents of the flask are then filtered through a Schleicher and Schüll filter, as above, washed, and ashed in a crucible as before. The weight obtained this time

represents the barium sulphate formed from the ethereal sulphates, from which the H_2SO_4 may be calculated, and by subtracting this weight from the one first obtained (total sulphates) the remainder obtained will represent the weight of the preformed sulphates. According to the revision of the atomic weights of the elements 100 parts of barium sulphate correspond to 34.29 parts of SO_3 or 1 part to 0.3429, and 41.99 parts of H_2SO_4 or 1 part to 0.4199.

Example: Suppose 80 milligrammes of barium sulphate were obtained in the first process, the total H_2SO_4 would be 80 times 0.4199, or 33.592 milligrammes in 50 c.c. = 67.18 in 100 c.c., or 0.6718 gramme per liter; since 100 $BaSO_4$ represents 41.99 H_2SO_4. Suppose 30 milligrammes were obtained in the second process, then the H_2SO_4 of the ethereal sulphates would be calculated as follows: 30 times 0.4199 = 12.597 milligrammes in 100 c.c. of urine or 0.126 gramme per liter.

☞ The difference between the H_2SO_4 of the total sulphates in this case and the H_2SO_4 of the etheral sulphates, 0.6718 minus 0.126 = 0.546, represents the amount of H_2SO_4 of the preformed sulphates in grammes per liter. Hence in this example the results are as follows:

H_2SO_4 of the total sulphates, ...0.6738 gm. per liter.
H_2SO_4 of the total sulphates, ...0.07 per cent. nearly.
H_2SO_4 of the total sulphates, ...0.317 grains per fluidounce.
H_2SO_4 of the ethereal sulphates, ...0.126 per liter.
H_2SO_4 of the ethereal sulphates, ...0.0126 per cent.
H_2SO_4 of the ethereal sulphates, ...0.057 grains per fluidounce.
H_2SO_4 of the preformed sulphates, .0.547 gm. per liter.
H_2SO_4 of the preformed sulphates, .0.055 per cent.
H_2SO_4 of the preformed sulphates, .0.257 grains per fluidounce.
Ratio of preformed H_2SO_4 to ethereal, 4.3 to 1.

If it is desired to calculate the sulphates as SO_3 divide the above figures by 1.23.

A more rapid method, attributed to Folin and described by Hawk, is the following: place 125 c.c. of urine in an Erlenmeyer flask; dilute with 75 c.c. of water, and acidify the mixture

with 30 c.c. of dilute hydrochloric acid. (1 volume HCl to 4 of water.) At room temperature add 20 c.c. of a 5 per cent. solution of barium chloride slowly, drop by drop, preferably from a dropper or capillary funnel made from an ordinary calcium chloride tube, delivering 10 c.c. in 2-3 minutes. Allow the mixture to stand one hour; then filter through a dry filter paper: (a double determination can be made by filtering through a quantitative filter paper or collecting on a Gooch crucible, using the filtrate for the ethereal sulphates and the residue for the preformed).

Boil 125 c.c. of the filtrate gently for, at least, half an hour; cool; filter; wash the precipitate on the filter with about 250 c.c. of water; dry it in an air bath; ignite in a crucible; cool; weigh.

In calculating, multiply the weight of the barium sulphate found by 2, since only one-half c.c. of the total volume of the fluid was precipitated by the barium chloride in the filtrate.

☞ **Quantitative Determination of the Preformed Sulphates.**—If for any reason it is desired to determine these separately, 25 c.c. of urine and 100 c.c. of water in a 250 c.c. Erlenmeyer flask are acidified with 10 c.c. of dilute HCl (1 to 4). Or, if the urine is of low specific gravity, 50 c.c. are used and 75 c.c. of water. Ten c.c. of a 5 per cent. barium chloride solution are added slowly as before without stirring or shaking; the mixture is allowed to stand at least an hour, then shaken up, filtered or collected on a Gooch crucible, ignited and weighed as before.

Quantitative Determination of Indican.—For clinical purposes a brilliant blue obtained in urine of specific gravity below 1020 is quantitatively significant. Askenstedt's method may be used if a quantitative analysis is desired, or the method of Ellinger already described.

Quantitative Determination of the Phenols.—The method of Kossler and Penny modified by Neuberg depends upon the precipitation of the phenols as triodophenol, according to the reaction $C_6H_5OH+6I=C_6H_2I_3OH+3HI$.

From any large quantity of urine, say, 500 c.c., acetone is removed by rendering the urine slightly alkaline and evaporating in this case to 100 c.c. The fluid thus obtained is acidulated with

sulphuric acid sufficient to form 5 per cent. of the original volume and repeatedly distilled. The individual portions thus obtained are shaken with calcium carbonate until the acid reaction has disappeared, so as to remove any nitrous or formic acid that may be present. The fluid is now again distilled and the distillate treated with a solution of one gramme of caustic soda and six grammes of lead acetate in substance. The mixture is kept on a boiling water-bath for about fifteen minutes. A portion of the lead oxide is thus dissolved by the phenol to form basic phenolates, while any aldehydes or ketones, that may have been formed from the small amount of carbohydrates that are present in every urine, escape. To remove these entirely, the mixture is heated over a free flame connected with a condenser until a few cubic centimeters of the distillate no longer reduce an alkaline solution of silver nitrate. After five minutes this point is usually reached. The fluid is then acidified with sulphuric acid as before and is distilled, water being added from time to time. The distillate is placed in a glass-stoppered bottle, treated with a decinormal solution of sodium hydrate until the reaction is markedly alkaline, and immersed in hot water. To the hot fluid a decinormal solution of iodine is added in an amount which should exceed that of the alkaline solution by 15 to 25 c.c. The bottle is now closed at once, shaken and set aside until cool. The solution is then acidified with dilute sulphuric acid and the excess of iodine which was not used in the formation of the tri-iodophenol retitrated with a decinormal solution of sodium thiosulphate. One c.c. of the iodine solution represents 1.567 milligrammes of phenol, or 1.8018 milligrammes of cresol. As the latter predominates in the urine, it is best to express the results in terms of cresol. Thus modified, the method is also applicable in the presence of sugar (Simon).

Colorimetric Determination of the Phenols.—Heat 50 c.c. of urine with 5 c.c. of strong hydrochloric acid in a casserole until reduced to half its volume; then add water 10 c.c.; place in a still and distill over a slow flame. The phenols only will pass over. The distillation should be conducted so as to get about 20 c.c. of distillate, which is then diluted so as to bring it up to 50

c.c. Take 20 c.c. of this liquid and add to it 20 drops of Millon's reagent and heat, adding if necessary 2 or 3 drops of nitric acid. When the maximum color is obtained, let cool and examine with Amann's chromometer. Tables which accompany the instrument will indicate the amount of phenols sought for.

This process is much simpler than the preceding one if the chromometer is available.

Quantitative Determination of the Neutral Sulphur Compounds. —The total sulphur of the urine is first determined by the method of Hoehnel, Glaser and von Asboth, modified by Modrakowski, as follows: From 1 to 2 grammes of sodium peroxide are placed in a nickel dish and drop by drop 50 c.c. of urine are added from a pipette. Evaporate the fluid on a water-bath until it becomes syrupy and then add slowly and with great care, while stirring, 2 to 3 grammes more of the peroxide. The reaction is at first violent but gradually subsides. The dish is then removed from the water-bath and heated over a small alcohol lamp, with addition if necessary of 1 to 3 grammes more of the peroxide. When the mass appears as a brown syrup and finally thickens the reaction is over. When cool, the fused mass is dissolved in hot water, filtered, rendered feebly acid with hydrochloric acid solution and the total sulphate determined as before.

In calculating the total sulphates remember that the solution obtained by oxidizing with the sodium peroxide, whatever its amount may be, represents 50 c.c. of urine; hence add hydrochloric acid in the proportion of 5 c.c. to 50 or more, and 10 per cent. barium chloride, in the proportion of 25 c.c. to 50 or more. Subtract the amount of barium sulphate obtained by the method given above, using another portion of the same 24 hours' urine, from the amount of sulphate obtained above by oxidizing all the sulphur of the urine, and the difference indicates the total unoxidized sulphur in terms of barium sulphate. Calculate the amount of oxidized sulphur and unoxidized sulphur by reckoning the molecular weight of barium sulphate to be 184.11, of which the sulphur having an atomic weight of 31.83 constitutes 17.29 per cent. Hence, if the amount of total sulphur were indicated by 85 milligrammes of barium sulphate and the amount of total sul-

phates by 80 milligrammes in 50 c.c. of urine, then the unoxidized sulphur would be represented by 5 milligrammes, $BaSO_4$, for 50 c.c. of urine, or 10 milligrammes for 100 c.c. In every 100 c.c. of the urine, then, there would be 1.73 milligrammes (17.29 per cent. of 10) of unoxidized sulphur, or 17.30 milligrammes per liter = 0.0173 grammes per liter.

The amount of unoxidized sulphur from 80 milligrammes of $BaSO_4$ in 50 c.c. of urine = 160 mg. in 100 c.c. would be 27.66 milligrammes (17.29 per cent. of 160) in 100 c.c. of urine = 0.277 grammes per liter.

The total sulphur, then, in this urine would be 0.277 plus 0.0173 = 0.2943 grammes per liter, of which the unoxidized represents six per cent. nearly.

In the **Osborn-Folin Method** 25 c.c. of urine, or 50 c.c. of very dilute urine, are placed in a nickel crucible and about 3 grammes of sodium peroxide added. The mixture is evaporated to a syrup over the steam water-bath and heated carefully for 15 minutes over an alcohol flame until it becomes solid. After cooling great care is taken to moisten the mass with 1 to 2 c.c. of water; 7 or 8 grammes more of peroxide are sprinkled over the contents and the whole is fused over the alcohol flame for about 10 minutes. Allow the crucible to cool for a few minutes; add about 100 c.c. of water to the contents, and heat at least one-half hour over an alcohol flame to dissolve the alkali and decompose the sodium peroxide. Next rinse the mixture into a 400-450 c.c. Erlenmeyer flask, by means of hot water, and dilute it to about 250 c.c. Heat the solution nearly to the boiling point and add 15-18 c.c. of concentrated hydrochloric acid slowly until the nickelic oxide, derived from the crucible, is just brought into solution. A few minutes' boiling should now yield a clear solution. In case too little peroxide or too much water was added for the final fusion a clear solution will not be obtained. In this event cool the solution and remove the insoluble matter by filtration.

To the clear solution add 5 c.c. of very dilute alcohol (about 18-20 per cent.) and continue the boiling for a few minutes. The alcohol is added to remove the chlorine which was formed when the solution was acidified. Add 10 c.c. of a 10 per cent.

solution of barium chloride slowly, drop by drop, to the liquid. Allow the precipitated solution to stand, in the cold, 48 hours, and then filter and continue as above under Total Sulphates (Hawk).

Other Methods.—The total sulphur may also be determined by the sodium hydroxide and potassium nitrate fusion method, as in determining the total phosphorus (see "Total Phosphorus"). After fusion, instead of acidifying with nitric acid, hydrochloric is used and the mixture evaporated on the water-bath, moistened with a few drops of dilute HCl, and brought into solution with hot water. After filtering, the filtrate is boiled and immediately precipitated by 10 c.c. of a 10 per cent. barium chloride solution, added slowly drop by drop. The precipitate is allowed to settle for two hours; filtered cold; ignited and weighed, as above. Calculation may be as SO_3 or as S.

QUANTITATIVE DETERMINATION OF THE TWO GROUPS OF NEUTRAL SULPHUR COMPOUNDS.

Remove the oxidized sulphur by precipitation with barium chloride as in the method for total sulphates; filter and add bromine water to the filtrate. Further, add barium chloride solution, and the easily oxidizable sulphur is precipitated as barium sulphate. Subtracting the amount obtained from that of the total neutral sulphur the remainder represents the "biliary" sulphur, i. e., that which is oxidized with difficulty.

Quantitative Determination of Cystine.—Alberhalden's method is as follows: The entire 24 hours' urine is filtered and the residue washed with ammonia. The filtered urine and the ammonia washings are united. 500 c.c. of the total,—preferably after concentration in a vacuum at 40° C. (104° F.),—are then treated with 4 c.c. of normal sodium hydroxide solution and shaken for from 6 to 8 hours with an ethereal solution of 4 grammes of beta-naphthalene sulphochloride. At intervals of one and a half hours 3 c.c. of the normal sodium hydroxide solution are further added. At the expiration of the shaking the ether layer is removed and the aqueous solution over-saturated with hydrochloric acid. The resulting precipitate is filtered off and after decolorization with animal charcoal crystallized from hot absolute alcohol. The crystals are dried at 100° C. (212° F.) and weighed. One gramme of the compound represents 0.35 gramme of cystine.

CHAPTER XII.

CERTAIN AROMATIC COMPOUNDS IN URINE: CONJUGATE GLYCURONATES, AROMATIC OXYACIDS, ALKAPTON BODIES, INOSITE.

The conjugate glycuronates; chemistry and source.
Drugs causing formation of glycuronates: camphor, chloral, etc.
Properties of the glycuronates:—phenyl-hydrazine combinations, etc.
Physiology of the glycuronates; origin, synthesis, quantity.
Clinical significance of the glycuronates: mistaken for sugar, etc.
Clinical tests for glycuronates: action on copper tests; the orcin test; test of B. Tollens with napthoresorcin; Neuberg's test-method.
The compound glycocolls: hippuric acid; phenaceturic acid.
Chemistry of hippuric acid: structural formula and properties.
Physiology of hippuric acid: synthesis, quantity, occurrence in the urine.
Physiological variations in the quantity of hippuric acid.
Clinical significance of hippuric acid; relation to diabetes and nephritis.
Clinical notes on relation of hippuric acid to other aromatics.
Clinical tests for hippuric acid; microchemical tests, etc.
Quantitative determination of hippuric acid: (1) weighing of the crystals and (2) method of H. D. Dakin, advised by Hawk.
Phenaceturic acid; structural formula, constitution, occurrence.
The aromatic oxyacids;—paraoxyphenylpropionic acid and hydro-paracumaric acid.
Structural formulas and chemistry of the oxyacids.
Physiology of the oxyacids;—source and quantity.
Clinical significance of oxyacids; relation to intestinal putrefaction.
Clinical colorimetric determination of the quantity of oxyacids.
Preparation of Millon's reagent.
The alkapton bodies; homogentisic and uroleucic acids.
Chemistry of homogentisic acid: structural formula, theoretical constitution and properties.
Physiology of homogentisic acid; relation to intestinal fermentation, etc.
Clinical significance of homogentisic acid; mistaken for sugar in urine.

Differentiation of homogentisic acid from pyrocatechin.
Clinical tests:—reduction of copper salts; ferric chloride test; the darkening with alkali and the author's hypobromite test.
Behavior of alkapton urine with various clinical tests.
Isolation of homogentisic acid.
Uroleucic acid;—properties.
Inosite: structural formula, theoretical constitution; properties.
Clinical significance of inosite.
Clinical tests for inosite:—Scherer's test; Gallois's test; isolation.

THE CONJUGATE GLYCURONATES.

Glycuronic acid, $CHO.(CH.OH)_4COOH$, occurs in urine in the form of conjugate glycuronates, *i. e.*, coupled with potassium sulphate, the glycuronate being formed either by combination with protein compounds, drugs or poisons. The drugs or poisons with which glycuronic acid may form coupled glycuronates are the following: arsenic, acetanilid, benzole, butylchloral hydrate, camphor, chloral hydrate, chloroform, curare, copaiba, hydroquinone, menthol, morphine, naphthaline, nitrobenzole, phenol, resorcin, salicylic acid, salol, sulphonal, turpentine and thymol. When camphor is taken campho-glycuronic acid occurs in the urine; chloral hydrate forms urochloralic acid (trichlorethyl-glycuronic acid); trichlorbutyl alcohol and butylchloral hydrate form trichlorbutyl-glycuronic acid; turpentine, turpenglycuronic acid; naphthalin, naphthol-glycuronic acid. The conjugate glycuronates are lævorotatory; only a few reduce cupric salts, etc. They do not form crystalline combinations with phenylhydrazine unless first boiled with a dilute mineral acid; they are not fermentable; they react with phloroglucin hydrochlorate but not with orcin. (Glycuronic acid itself is dextrorotatory, reduces cupric salts, etc., and forms a crystalline combination with phenylhydrazine.)

Physiology.—Glycuronic acid probably originates in the tissues and is an intermediate product of glucose metabolism. The synthesis of the conjugate glycuronates is effected in the liver. The normal quantity in the urine is 25 milligrammes per 100 c.c. The drugs mentioned above increase the quantity. If the aromatic glycuronates are increased in amount the excretion of indoxyl sulphate, etc., is correspondingly diminished.

Clinical Importance of the Glycuronates.—The points of importance to be remembered about the glycuronates are as follows:

(1) Reduction by them of the alkaline cupric solutions may mislead the physician into belief that sugar (glucose) is present in the urine.

(2) Recognition of the glycuronates may lead to the suspicion and detection of poisoning by chloral, etc.

(3) The finding of much indoxyl (indican) together with the aromatic glycuronates in marked degree is indicative of a high degree of intestinal putrefaction, which may explain toxic phenomena previously not understood.

It was formerly thought that the glycuronates were in relation to diabetes mellitus, but this idea has been abandoned, since considerable quantities may occur in the urine of healthy persons presenting neither the history nor the clinical features of diabetes mellitus.

Clinical Tests for Glycuronates.—Care must be taken not to mistake pentoses for glycuronates. The former are of but little clinical significance.

1. The presence of glycuronates may be inferred if the urine *reduces Haines' or Fehling's solutions,* while at the same time there is a history of ingestion of any of the drugs mentioned above. Such urine is not fermented by yeast when, for example, Einhorn's or Lohnstein's apparatus is used (see "Sugars").

2. Glycuronates affect the *orcin test* as follows: to the urine is added an equal volume of fuming hydrochloric acid (specific gravity, 1.19) and a pinch of orcin. At first no precipitate occurs, but after long boiling—several minutes or more—a greenish gray precipitate appears if glycuronates are present. Pentoses give an immediate reaction.

As Tollens says, the older methods are tedious and may give faulty results, hence the following is recommended by him.

3. **Test of B. Tollens.**—This test depends upon the fact that in the presence of glycuronic acid naphthoresorcin and hydrochloric acid give rise to a blue ether-soluble coloring matter showing a distinct dark band in the sodium line. (The pentose-naphthoresorcin blue coloring matters are insoluble in ether.)

The test is applied as follows: Naphthoresorcin (a piece the size of a millet seed) is added to 5 c.c. of hydrochloric acid, specific gravity 1.19, and to this 5 c.c. of urine added and the whole boiled. When this occurs hold the tube over a small flame for one minute. Four minutes later cool under the hydrant and shake with an equal volume of ether. When this has separated the ether will appear from dark blue to violet, according to the amount of glycuronic acid present. If the amount is small, the color will be a light reddish-violet. Spectroscopically a dark band will appear in the sodium line. In urines containing sugar (dextrose) if it is desired to test for glycuronates the sugar must first be removed by fermentation.

Laboratory Note.—In using the cupric tests for sugar a reaction occurring after the tube cools should suggest the possibility of the presence of the glycuronates in abnormal amount, and then careful application of the fermentation test for sugar and of the Tollens' test for glycuronates should be made. If fermentation is positive glucose or levulose is present; if fermentation is negative and Tollens' test negative, suspect pentoses and try the orcin test. If fermentation is negative and Tollens' test positive, glycuronates are the cause of the reduction of the sugar-test liquid. For a delicate test, however, see the next paragraphs.

4. **Neuberg's Test Method.**—Heat urine in an autoclave for 1-2 hours at 115° C. with from 1-2 per cent. of sulphuric or phosphoric acid. Cool, neutralize with sodium carbonate, immediately acidify with acetic acid, filter and to 250 c.c. of the filtrate add a hot aqueous solution of 5 grammes para-brom-phenylhydrazine hydrochlorate and 6 grammes of sodium acetate. If a cloudiness appears, continue warming and it will disappear; in 5-10 minutes needle-shaped crystals form. Let cool and filter off the crystals. Heat the filtrate and get second crop of crystals. Repeat this process 4 or 5 times. Collect all the crystals, wash with water and absolute alcohol and recrystallize several times in 60 per cent. alcohol. The melting point of the pure crystals is 230° C. Two centigrammes of the crystals in a solution of 4 gm. pyridine in 6 c.c. absolute alcohol is lævorotatory 7° in the 10 cm. tube.

5. **Guido Goldschmiedt's Reaction.**—To ½-1 c.c. of urine add 2 drops of a 15 per cent. alcoholic alpha-naphthol solution. Mix well and float upon 3-4 c.c. of concentrated sulphuric acid. At the juncture appears a violet ring which, on standing, extends in width on the side of the urine while the sulphuric acid assumes a greenish coloration. Fractions of a milligramme may be detected by this test. The reaction is slow. It is well to obtain a negative reaction for nitrates and nitrites with the diphenylamine test before making the alpha-naphthol test.

☞ The alpha-naphthol reaction of Guido Goldschmiedt is said by Mayerhofer to be well adapted for the early recognition of intestinal putrefaction in nurslings. Glycuronic acid can nearly always be demonstrated in the urine of abnormally nourished infants, though the indican reaction is most often negative in such cases.

☞ **Author's Modification of Goldschmiedt's Test.**—For a rough quantitative test the author uses the alpha-naphthol reaction as follows: mix two drops of the 15 per cent. alcoholic solution of alpha-naphthol with 1 c.c. of the urine. Then pour in 3 c.c. of strong pure sulphuric acid. Mix well and dilute the mixture with 15 c.c. of distilled water. Mix well by shaking. The depth of the violet color roughly indicates the amount of glycuronate relatively present. Let stand and after a while a dark-violet precipitate in flocculi separates. Sediment in the centrifuge and the bulk of this precipitate may be calculated in a Purdy percentage tube. One per cent. by bulk of the precipitate is an abundance, urines containing but little glycuronate showing no measurable amount. Or titrate with barium chloride solution (20 per cent.) until color disappears.

THE COMPOUND GLYCOCOLLS.

Glycocoll, CH_3COOH, resembles sulphuric acid and glycuronic acid in that it combines with certain aromatic radicals to form compounds which appear in the urine. These compounds are known as the *compound glycocolls,* the most important of which are *hippuric acid* and *phenaceturic acid.* It also combines with certain drugs and poisons belonging to the aromatic group, as salicylic acid, nitrobenzoic acid, furfurol, toluol, etc.

HIPPURIC ACID.



This acid represents the combination of benzoic acid with glycocoll and may be deemed a substitution product of ammonia, in which two hydrogen atoms are replaced by the radicals of benzoic acid and acetic acid respectively. Boiled with an alkaline hydroxide or mineral acid it yields benzoic acid and glycocoll, and the same change takes place in urine which has undergone ammoniacal decomposition, especially when albumin is present.

Chemistry.—Hippuric acid, $C_9H_9NO_3$, is also known as benzoyl-glycocoll and benzoyl-amino-acetic acid. In the pure state it is crystalline, forming vertical rhombic prisms if typical. In the urinary sediment, however, these crystals may also appear as fine needles, four-sided prisms or pillars whose ends terminate in two or in four planes. The crystals may show indentations. The crystals are not readily soluble in cold water, but are easily soluble in hot water, hot alcohol and in ether. The solutions are all strongly acid.

Physiology.—The synthesis is probably effected in the kidney. It is derived from the food. Its origin is the oxidation of proteins. Its quantity depends upon the degree of protein decomposition in the bowel. It occurs in the urine almost always in solution, rarely in the sediment. It sometimes is a constituent of concretions. The total quantity in the urine ranges from 0.1 to 1 gramme and is *increased* by vegetable diet and certain fruits, as prunes, mulberries, cranberries, blueberries; and by the ingestion of any substance containing the benzoic acid radical, as benzoic acid, cinnamic acid, salicylic acid, phenylpropionic acid, kinic acid, oil of bitter almonds, toluol, or benzylamine. The quantity is *decreased* by a meat diet, but even on an exclusive meat diet small quantities of it may be found in the urine.

Clinical Significance.—Hippuric acid is *increased* in acute febrile diseases, hepatic diseases, diabetes mellitus and chorea. It is decreased in nephritis and in amyloid kidney.

Clinical Notes.—In nephritis the power of the kidneys to unite benzoic acid with glycocoll is absent, hence it has been proposed to test the secretory ability of the kidneys by testing their power to transform benzoic acid into hippuric.

Hippuric acid is most likely to be deposited in the sediment of the urine after the person has been eating heartily of cranberries. Care must be taken not to confound the crystals of hippuric acid with those of ammonio-magnesium phosphate, the latter occurring in feebly acid or alkaline urine.

The significance of hippuric acid is much the same as that of the other aromatics; if present in quantities around one gramme per 24 hours excessive protein decomposition in the bowel may be inferred.

Clinical Tests for Hippuric Acid.—When it occurs as a urinary sediment or in concretions, the crystals may be tested by their solubility: hippuric acid is soluble in 600 parts of cold water, easily soluble in hot water, hot alcohol and ether, but insoluble in petroleum-ether and benzene; soluble in ammonia and insoluble in hydrochloric acid. The latter serves to differentiate the crystals from those of triple phosphate, which have much the same microscopical appearance, but are readily soluble in hydrochloric acid.

Micro-chemically, if the urine contains an excess of hippuric acid, the latter may be detected by evaporating the urine to about one-fourth its volume and feebly acidulating with hydrochloric acid. On standing a few hours the acid crystallizes out and may be recognized *with the microscope* by the prismatic form, the prisms often showing indentations, and *chemically* by insolubility in hydrochloric acid and solubility in ammonia.

Chemically, if the urine be evaporated to dryness with concentrated nitric acid and the residue heated in a test-tube, hippuric acid or hippurates may be recognized by the odor of oil of bitter almonds, due to formation of nitrobenzole.

Quantitative Determination of Hippuric Acid.—The amount of hippuric acid is determined as follows: 100 c.c. of the urine are made slightly alkaline with sodium carbonate and evaporated to a thick syrup. To this are added 500 c.c. of alcohol (90-95 per

cent.) and the mixture allowed to stand 24 hours. It is then filtered and the alcohol is distilled off. The aqueous solution remaining is acidulated with dilute sulphuric acid and the liberated hippuric acid extracted with acetic ether, using several portions. The ethereal solution is evaporated to dryness and washed with petroleum-ether. The residue is dissolved in warm water and evaporated at a temperature of 50°-60° C. (122°-140° F.). On cooling the crystals are obtained, carefully dried on plaster of Paris plates, shaken with benzole or petroleum ether and weighed. To the amount obtained is also added the weight of



FIG. 17.—Soxhlet Extraction Apparatus.

what crystals may be obtained by extracting the liquid left (after the first crop of crystals has been obtained) with acetic ether and evaporating. The crystals are milk-white and semi-transparent, melting at 187.5° C. (369.5° F.) and responding to the test with nitric acid.

Like all processes involving crystallization, the above is only approximate in results, and for close work can not be depended upon, hence Hawk advises use of H. D. Dakin's methods, which consist of preliminary treatment to remove urea and subsequent

volumetric or gravimetric determination. One hundred and fifty c.c. of urine are evaporated almost to dryness; 1 gramme of di-acid sodium phosphate and 25 grammes of gypsum added; the mixture rubbed up with a pestle and stirred with a spatula until uniform; dried in a water oven for two hours; again rubbed well, transferred to an extraction shell, and extracted in a Soxhlet apparatus (Fig. 17) with ethyl acetate over a sand bath for two hours. The acetate extract is transferred to a separatory funnel and the original flask rinsed with fresh ethyl acetate enough to make the total volume in the funnel 100 c.c. The acetate solution is washed five times with a saturated solution of sodium chloride, using 8 c.c. of the NaCl each time, shaking vigorously and removing entirely before adding fresh chloride. The hippuric acid is then freed from urea and is determined volumetrically by transferring to a Kjeldahl flask, adding 25 c.c. of water and a small piece of pumice, attaching to a condenser and distilling off the ethyl acetate. The nitrogen in the residue is then determined by the Kjeldahl calculation: 1 c.c. of n/10 H_2SO_4 = 0.0179 gramme of hippuric acid. Phenaceturic acid and indolacetic acid contribute a slight error, since their nitrogen is determined at the same time.

PHENACETURIC ACID. $CH_3.CO.NH.CH_2.COOH.$



This substance, $(C_6H_5.CH_2.CO)NH.CH_2.COOH$, may be regarded as phenylacetyl glycocoll, and results from the combination of phenylacetic acid with glycocoll. It occurs in very small quantities in human urine, and may be obtained by evaporation of the solution obtained by shaking the hippuric acid crystals with petroleum ether. The crystals are small rhombic plates with rounded angles, much resembling those of uric acid, melting at 143° C., assuming a reddish color and emitting an aromatic odor when heated above this point. The clinical significance is that of the aromatics in general.

THE AROMATIC OXYACIDS.

The two oxyacids most commonly present in urine are paraoxyphenylpropionic acid, $CH_2CH_2.COOH$, also known as hydro-



paracumaric acid and paraoxyphenylacetic acid, CH_2COOH, the



latter being an oxidation product of the former. Both are tyrosine derivatives.

Physiology.—The oxyacids are formed during the process of intestinal putrefaction. They range from 0.01 to 0.02 gramme in the urine of 24 hours. When tyrosine is ingested, *paraoxyphenyllactic acid* is also found in the urine. *Pathologically* in acute yellow atrophy of the liver and in phosphorus poisoning *paraoxyphenylglycolic acid* and *oxyamygdalic acid* may occur along with leucine and tyrosine.

Pathology.—The oxyacids are increased by circumstances which favor intestinal putrefaction and are also increased in carbolic poisoning.

Isolation and Clinical Determination With the Chromometer.—The oxyacids may be isolated and clinically determined as follows: 50 c.c. of urine with 5 c.c. of strong hydrochloric acid are heated in a casserole and concentrated to one-half. Care should be taken during this process to secure abundant ventilation, as the odor evolved is unpleasant. The salts of the oxyacids are decomposed into free oxyacids and the bases converted into chlorides. The conjugate sulphates are also decomposed into phenols, indols, etc., and a part of the phenols are volatilized. The liquid is allowed to cool when it is poured into graduated test-tube marked No. 1. To it are added 10 c.c. of ether and the tube is rotated or shaken for 15 minutes, so as to dissolve the oxyacids, phenols and indols. The ether is then removed by means of a pipette into another tube marked No. 2. Tube No. 1 is allowed to stand still longer, and from time to time all the ether rising is

pipetted from it to tube No. 2. Phenols are next removed by adding to the contents of No. 2 five c.c. of a decinormal solution of sodium hydroxide and 10 c.c. of distilled water, after which the tube is rotated or shaken for 15 minutes as before. The phenols and indols remain in the ethereal solution while the oxyacids unite with sodium and are in aqueous solution, hence the ether is removed by pipetting and cast aside. The remaining aqueous solution is acidulated with 15 c.c. of decinormal sulphuric acid which sets free the oxyacids. Add 10 c.c. of ether and rotate or shake the tube as before. Pipette off the ethereal solution, evaporate it in a casserole, and dissolve the residue in 20 c.c. of distilled water. Then add 20 drops of Millon's reagent, heat until the maximum red color is obtained and if convenient examine with Amann's chromometer. Tables which accompany the instrument show the quantity of the oxyacids sought for by colorimetric dosage. The chromometer must be imported.

Laboratory Note.—Millon's reagent may be made as follows: (1) Dissolve one part of mercury in two parts of ordinary nitric acid. Evaporate to one-half its volume and add 1½ parts of water. After 24 hours decant the clear supernatant fluid.

(2) It may be more conveniently made by dissolving a few grammes of mercuric nitrate in an amount of water just sufficient for its solution and further dissolving any basic salt which remains with fuming nitric acid (specific gravity 1.486 to 1.500), and adding drop by drop a solution of sodium acetate until the reagent gives a red color on boiling with a few drops of dilute solution of phenol.

ALKAPTON BODIES: HOMOGENTISIC AND UROLEUCIC ACIDS.

In rare cases the urine darkens on standing. The phenomenon is sometimes due to the presence of certain aromatic oxyacids to which such names as pyrocatechuic, urrhodinic, glycosuric, uroxanthinic and uroleucic or uroleucinic acids have been given. It is likely, however, that the first four of these are all identical with *homogentisic* (*homogentisinic*) *acid,* and that the peculiar reaction obtained in alkapton urines are due to this agent.

Chemistry.—Homogentistic acid  is regarded as a normal intermediary product in the destruction of tyrosine and phenylalanine, but its appearance in the urine is an expression of metabolic insufficiency, which renders the destruction of the benzene ring impossible.

Urine containing homogentisic acid stains the clothing (linen) dark, and on standing darkens from above downward, until, finally, the whole appears like molasses,—so called "Black urine."

Alkalies hasten the darkening, while acids dissolve the color. Homogentisic acid reduces the alkaline cupric solutions but does not affect Nylander's reagent characteristically, *i. e.*, not like glucose, merely darkening in time from the action of the alkali. Silver nitrate is reduced in the cold. Fermentation is negative and no crystals are formed with phenylhydrazine. Optically homogentisic acid is inactive.

☞ **Physiology.**—The occurrence of the alkapton bodies in the urine merely represents an unusual form of intestinal putrefaction, not necessarily such as to affect the health. The condition appears to be congenital, runs in families, is present in the offspring of consanguineous marriages, but is seldom hereditary. It resembles, therefore, albinism and cystinuria. The amount reckoned by reducing power ranges from 3 to 7 grammes in 24 hours, is slightly lessened by a vegetable diet, and reduced one-half by hunger. The condition, originally discovered by Boedecker and in this country by Marshall, is extremely rare, only about 50 cases having been reported. The writer has described one case in the *Medical Record,* May 21st, 1910, besides which not half a dozen cases have been recognized in this country, so far as the author is aware at present writing.

Clinical Significance.—The importance of alkaptonuria clinically, lies chiefly in the error caused by the reduction of the alkaline cupric solution, resulting in rejection for life insurance and treatment by dietetic regulations for diabetes mellitus, when the

condition, so far as known, is a harmless one and unaffected by restriction of carbohydrates.

In the author's case there had been a long history of bowel trouble, beginning with typhoid fever; indicanuria was also a notable feature when the patient was first examined.

Differentiation from pyrocatechinuria may be difficult, but absence of history of ingestion of phenols and the rapid disappearance of the green-blue color with ferric chloride in testing the urine point to alkaptonuria. It is probable that most urines supposedly rich in pyrocatechin are really cases of alkaptonuria.

Clinical Tests.—The behavior of homogentisic acid with various reagents in urine analysis has been shown by the author (*Medical Record,* May 21, 1910).

In testing for sugar much that was interesting developed, as follows: Four c.c. of Haines' solution were boiled and the alkapton urine added, drop by drop, with boiling after each drop: as soon as the first drop struck the boiling reagent, the latter immediately darkened throughout its upper third. After four or five drops had been added, the entire liquid darkened, and when the sixth drop was added, a yellowish precipitate suddenly formed, which turned somewhat reddish in a few minutes. On repeating the experiment, the same precipitate formed when the sixth drop was added. Five c.c. of Fehling's solution were next boiled, and the same urine added, drop by drop, as before. The same darkening of the fluid was noticed as in the case of Haines' solution, but it required 22 drops to produce the yellowish precipitate. Ten c.c. of the alkapton urine were next boiled several minutes with Nylander's bismuth test reagent. No characteristic change was observed other than the darkening due to the alkali. Ten c.c. of the urine were then placed in an Einhorn saccharimeter with one gramme of condensed yeast and allowed to stand at a room temperature for 24 hours. No perceptible evolution of gas took place.

☞ The behavior with ferric chloride solutions was characteristic of homogentisic acid. When to 5 c.c. of alkapton urine a 20 per cent. solution of ferric chloride was added drop by drop a dark green color was noticed just as the drop of reagent struck

the urine, which immediately disappeared and left a slaty-grey precipitate of phosphates in its place. The same happened when a five per cent. solution of ferric chloride was used.

When Ehrlich's diazo reaction was sought for in this alkapton urine, an interesting result was observed. Ammonia being floated upon the sulphanilic acid-nitrite-urine mixture a double ring was obtained; below, a yellowish white ring of deposited phosphates, and just above it a slender, wavy, dark-brown ring, due to the action of the alkali upon the alkapton bodies. The slender brown ring soon disappeared. On shaking gently the mixture, the upper portion was gradually darkened by the ammonia.

It would appear that the alkapton bodies do not interfere with the oxidation of indoxyl to indigo blue.

Quantitative Tests.—The acidity of the specimens examined was normal and the ordinary method of titration with decinormal sodium hydroxide agreed with Folin's method, in which potassium oxalate is added to the urine with shaking. Phenolphthalein is reddened by sodium hydroxide in such urine, apparently, as in the case of normal urines, although the end-reaction is not quite so sharp as usual. Nothing of special interest was observed in the quantitative determination of urea by the Doremus instrument with the hypobromite solution, nor in that of phosphoric anhydride with uranium nitrate. In the quantitative determination of uric acid by the Folin method of titration with permanganate, the usual pink color of the permanganate was affected, at first being much more brownish than in the case of the normal urines. In the determinations of the chlorides by the Volhard-Luetke method much difficulty was encountered, and it was during this process that the writer's attention was first directed to the peculiar feature of this urine. In using the Luetke method the chlorides are indirectly determined by titration of the excess of silver nitrate in the silver-urine mixture by means of a solution of ammonium sulphocyanate. The end reaction is extremely sharp, but in the alkapton urine the end reaction was faint and indefinite until what was obviously an excess of the reagent was added.

☞ **Special Test for Homogentisic Acid.**—The most strik-

ing way to demonstrate the presence of homogentisic acid was found by the writer to be the following: the urine in small amount (1 c.c.) was carefully floated on 5-10 c.c. of a solution of sodium hypobromite (NaBrO) the same as used for determining urea with the Doremus instrument. Brown foam appeared on the surface of the mixture, and a dense brown band of color below the foam. Normal urines give a white foam and little or no color below the foam.

☞ Summary. Urine containing the alkapton bodies shows the following features:

1. It darkens gradually on exposure to the air from above downwards. The upper portion of a given sample may appear black as molasses while the rest of the liquid is only slightly affected. Finally, the entire body of fluid turns a dark brown or black. Addition of ammonia or other alkali hastens the change. Addition of hydrochloric acid removes the dark color.

2. If carefully floated in a test tube on the alkaline hypobromite solution used for the estimation of urea, it causes a brown-streaked foam to rise and a dense brown color band is seen below the foam.

3. Added drop by drop to boiling Haines' or Fehling's solutions, darkening immediately occurs and ultimately a greenish or yellowish red precipitate, simulating the presence of glucose. With Nylander's bismuth test reduction does not take place.

4. Fermentation tests are negative.

5. It gives a dark brown ring in Ehrlich's diazo test.

6. It interferes to a certain extent with the determination of uric acid with permanganate, and renders the end-reaction doubtful in the Volhard-Luetke indirect method of determining chlorides in the urine.

7. It does not appear to affect the nitroprussiate test for acetone, nor the usual tests for indican.

8. A characteristic test depending on the presence of homogentisic acid is the dark green color on addition of ferric chloride, which color immediately disappears.

Isolation.—Homogentisic acid may be isolated from the urine

as follows: Heat the entire 24 hours' urine to nearly the boiling point; add 5 grammes of neutral lead acetate for every 100 c.c. of urine, and as soon as the lead salt is dissolved filter off the precipitate and set aside the filtrate in the cold for 24 hours. Acidular almost colorless crystals of lead homogentisate are formed, which may be collected on a filter, dissolved in hot water, and tested as above described. Microscopically the crystals are in rosettes and stars and may appear deeply colored.

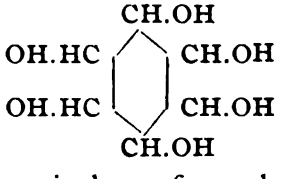
UROLEUCIC ACID.

This acid, also called dioxyphenyl-alpha-lactic acid, is also found in alkaptonuria, but less commonly than homogentisic. It reduces the alkaline cupric solutions and also Nylander's solution, if in quantity, 0.5 per cent. or more, but does not, it is said, give the green-blue color with ferric chloride solution.

INOSITE.

This substance, erroneously regarded as a carbohydrate, is really an aromatic.

Chemistry.—Its formula is $C_6H_6(OH)_6$, hexahydroxybenzole, structurally



When pure it occurs in large, fine, colorless, monoclinic or clinorhombic tablets, but impure it crystallizes in cauliflower-like masses. Its melting point is 217° C. (422° F.). It is soluble in water, dilute alcohol, and in acetic acid, dilute or concentrated, but insoluble in absolute alcohol or ether. It does not reduce the metallic oxides in alkaline solution, does not ferment, is optically inactive and does not combine with phenylhydrazine. Fermented with putrefying albumin it yields sarcolactic acid and is decomposed by Bacterium lactis with the formation of lactic acid, and subsequently yields butyric acid. It has a sweetish taste.

and when boiled with alkaline cupric solutions sometimes gives a greenish precipitate, which redissolves on cooling.

Physiology.—Its origin in the body is unknown.

Clinical Significance.—It occurs in normal urine after ingestion of large quantities of water. Pathologically it occurs in the last stage of chronic nephritis (subacute glomerular and interstitial), in tuberculosis, syphilis, typhus fever, diabetes mellitus and insipidus.

Clinical Tests.—The acid urine must be freed from albumin by boiling, cooling and filtration. Nitric acid oxidizes inosite to rhodizonic acid, which property may be utilized as a test for inosite as follows: (1) Scherer's test: on evaporating a solution containing inosite on the water bath nearly to dryness in a porcelain dish, having added a few drops of nitric acid, and then treated the nearly dry residue with some drops of freshly prepared, not too dilute, watery solution of ammonia and calcium chloride solution,—a rose-red mass remains, which, after some time, becomes changed in color. (2) Gallois's test: an aqueous solution evaporated to a few drops and treated in a porcelain dish with a little of Millon's reagent—a drop will suffice—gives a yellowish precipitate. If this is spread out on the edge of the dish quickly, and heated carefully, it becomes a dark red. The color disappears on heating. Starch, lactose, mannite, glycogen, uric acid, urea, taurine and cystine do not give this red, but albumin does, and it is said that sugar and tyrosine also interfere with the reaction.

Isolation.—In order to prepare the substance from urine for the purpose of recognition by the above tests, the following method will be found useful: a large quantity of albumin-free urine (several liters) is feebly acidified with acetic acid and concentrated to one-fourth its bulk on the water bath, precipitated completely with neutral acetate of lead, avoiding excess, filtered, and the warmed filtrate treated with basic acetate of lead, as long as a precipitate forms. After standing for forty-eight hours this precipitate is filtered off, washed, suspended in water, and treated with a stream of sulphuretted hydrogen. From the filtrate after some hours, uric acid separates, from which the fluid is poured

off. The solution is then evaporated to a syrup on the water bath and precipitated while boiling with three or four times its volume of absolute alcohol.

If a heavy precipitate results that rapidly settles, the hot alcoholic solution is simply poured off, but if a flocculent non-adhesive precipitate occurs, the hot solution is filtered through a heated funnel and allowed to cool. If, after 24 hours, groups of inosite crystals have deposited they are filtered and washed with a little cold alcohol. In this case it is advisable to dissolve the precipitate once more in as little boiling water as possible, and to precipitate it a second time with 3 or 4 volumes of absolute alcohol in order to avoid any loss of inosite. If, however, no inosite crystals separate, ether is added gradually to the clear cold alcoholic filtrate until a milky cloudiness results on shaking thoroughly, and it is then allowed to stand 24 hours. Almost all the inosite present then separates in the form of shining pearly leaves.

CHAPTER XIII.

THE NON-NITROGENOUS ORGANIC ACIDS.

Oxalic acid; occurrence and solubility in the urine.
Sources of oxalic acid;—amount for twenty-four hours, derivation.
Physiological variations in the amount of oxalic acid.
Pathology and clinical significance;—relation to neurasthenia and dyspepsia.
Clinical notes on "oxaluria."
Quantitative determination of oxalic acid;—the Dunlop-Baldwin method; the Salkowski-Autenrieth method.
Oxaluric acid.
Volatile fatty acids;—formic, acetic, etc.; clinical significance.
Quantitative determination of the volatile fatty acids.
Lactic acid (para-); occurrence and significance.
Isolation of lactic acid; method of Araki.
Succinic acid.

Various organic acids not containing nitrogen occur physiologically in the urine. These are oxalic, lactic and the volatile fatty acids (formic, acetic, etc.).

OXALIC ACID.

$$\begin{array}{l} COOH \\ | \\ COOH \end{array}$$

A minute quantity of this acid, $H_2C_2O_4$, occurs in all urine, in combination with calcium, forming calcium oxalate. This substance usually is held in solution by the diacid-phosphate of sodium, but at times appears in the sediment as minute octahedral crystals, disks, dumb-bells and spheres. Calcium oxalate is also a constituent of concretions.

Physiology.—The chief source of oxalic acid is the carbohydrates in the food, but a small amount appears to be derived from the tissues. It is intermediate between urea and uric acid and is either a product of the incomplete oxidation of uric acid or else formed from glycocoll and creatin. The amount in the

urine ranges from 20 to 35 milligrammes per 24 hours and is due to excessive carbohydrate decomposition in the body, as it is probably an intermediate product in the metabolism of carbohydrates.

The amount is *increased* by the ingestion of certain fruits and vegetables, especially rhubarb; also by apples, bananas, tomatoes, grapes, asparagus, spinach, beans, artichokes, beets, honey, potatoes and strawberries; by administration of uric acid, rhubarb, senna, and squill. Tea, coffee, cocoa, waters rich in lime salts, and effervescing drinks increase it.

Pathology.—The amount is *increased* in digestive and respiratory diseases, in neurotic conditions, in diabetes mellitus and icterus; in tuberculosis, leukemia, pernicious anemia, gout, and hepatic diseases. Independently a condition occurs known as "oxaluria," in which symptoms of dyspepsia and neurasthenia are observed. (See "Clinical Notes.")

Clinical Notes.—In diabetes mellitus the amount of oxalic acid may rise as high as 1.5 grammes in 24 hours when the sugar diminishes (vicarious oxaluria).

The so-called "oxaluria" is a condition in which calcium oxalate crystals are found in number in high colored urine of high specific gravity. In such cases symptoms of dyspepsia and neurasthenia may be present, perhaps due to deficiency of hydrochloric acid in the gastric juice with increased intestinal fermentation. The condition was formerly claimed to be an independent disease (false Bright's disease) and even now is regarded with suspicion by certain life insurance companies who connect metabolic disturbances with the etiology of nephritis. No doubt, however, that long continued excretion of the crystals may lead to irritation of the kidneys, producing albuminuria and cylindruria and grave nervous disturbances, and perhaps leading to calculous formation.

The clinical significance of increased oxalic acid elimination is probably slight, indicating merely incomplete metabolism in perhaps hepatic derangement, though of late it has been claimed that certain nervous diseases are due to it, just as in the past uric acid was held responsible.

A copious oxalate sediment does not necessarily signify an increase in oxalic acid; it is very common during the asparagus and rhubarb season.

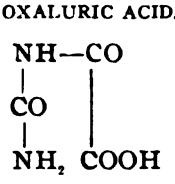
Quantitative Determination of Oxalic Acid.—(1) The Dunlop-Baldwin method is as follows: to 500 c.c. of the mixed 24 hours' acid or acidulated urine add a little thymol and 150 c.c. of 95 per cent. alcohol and allow the mixture to stand 48 hours in order that the oxalic acid may be completely precipitated as calcium oxalate crystals. At the end of the period collect the crystals on a filter, thoroughly wash with hot and cold water and with 1 per cent. acetic acid. Place filter in a small beaker and soak in a small amount of dilute hydrochloric acid. Wash with hot water until there is no further acid reaction. Filter the washings, evaporate to about 20 c.c. and add to the solution in hydrochloric acid. Add a very little calcium chloride solution to insure an excess of calcium.

Neutralize the solution of hydrochloric acid with ammonia and slightly re-acidulate, this time with acetic acid. Add strong alcohol, in amount one-half of the whole fluid volume, add a little thymol, and set the solution 48 hours. Collect the sediment on an ashless filter, wash with *cold* water and dilute acetic acid until the washings no longer react with silver nitrate, when acidulated with nitric acid. Incinerate the filter in a platinum crucible and heat first at a dull red and finally with a blast flame until it no longer loses weight. The ash is calcium oxide, 56 parts of which correspond to 90 of oxalic acid; hence multiply the weight of the calcium oxide by 1.6 to obtain the amount of oxalic acid in 500 c.c. of urine.

(2) Another method is that of Salkowski-Autenrieth as modified by Barth. The entire 24 hours' urine is placed in a precipitating jar, an excess of calcium chloride added, the urine rendered strongly ammoniacal, stirred well and let stand 18-20 hours. The precipitate is filtered off, washed with a small amount of water and dissolved in 30 c.c. of a hot 15 per cent. solution of hydrochloric acid. Using a separatory funnel (Fig. 17) the solution is extracted with 150 c.c. of ether containing 3 per cent. of alcohol, the extraction being repeated 4 or 5 times with fresh portions of

ether. The ethereal extracts are united, allowed to stand an hour in a flask, and then filtered through a dry filter. To the filtrate are added 5 c.c. of water and the ether distilled off. Charcoal may be used to decolorize, if necessary. The filtrate is concentrated down to from 3 to 5 c.c.; a little calcium chloride solution added, the solution made ammoniacal, and after a few minutes acidulated slightly with acetic acid. The acidified solution is allowed to stand two or three hours, the oxalate collected in a washed filter, washed, incinerated strongly and weighed. The amount of calcium oxide obtained multiplied by 1.6071 represents the amount of oxalic acid in the 24 hours' urine.

OXALURIC ACID.



Oxaluric acid exists in solution in the urine as ammonium oxalurate and is not found in sediments. It is present in very small quantities and is intermediate in composition between oxalic acid and urea.

VOLATILE FATTY ACIDS.

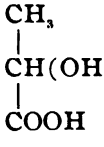
Normal urine contains traces of free formic, acetic, propionic and butyric acids, presumably the product of carbohydrate fermentation and, in lesser degree, of albuminous putrefaction. The amount in total is from 8 to 50 milligrammes per 24 hours, but in decomposing urine they are formed in larger quantity, which fact is probably due to the bacterial decomposition of the small amount of carbohydrate present. This is particularly true of the stale urine of diabetics.

Clinical Significance.—The amount is also increased by vegetarian diet, in fevers, acute yellow atrophy of the liver, phosphorus poisoning and diabetes mellitus. The term *lipaciduria* is applied to the pathological condition in which these acids are increased. In some cases valerianic acid and oleic acid have been found, the former in typhoid, variola, and acute yellow atrophy and the latter in phosphorus poisoning.

Clinical Notes.—The significance of the increase of fatty acids in diabetes is the same as that of the acetone bodies; in typhoid, variola, and acute yellow atrophy of the liver (valerianic acid) the significance is probably the same as that of leucine.

Quantitative Determination of the Volatile Fatty Acids.—The entire urine for 24 hours is collected, acidified with phosphoric acid in the proportion of 1 to 10, and distilled in a current of steam until the distillate is no longer acid. The distillate is then neutralized with sodium hydroxide solution, evaporated to dryness and the residue repeatedly extracted with absolute alcohol. The alcoholic extract contains sodium salts of the fatty acids, sodium benzoate and paracresol. The benzoate can be removed by evaporating the alcoholic solution to dryness, treating the residue with cold sulphuric acid, filtering and allowing the filtrate to stand until all the benzoic acid crystallizes out. The crystals can be filtered off and the filtrate neutralized with sodium hydroxide and shaken out with ether; the latter takes up the paracresol. The ethereal solution of paracresol is removed in a separatory funnel, the watery residue heated to drive out the last traces of ether, and the fatty acids determined in the aqueous solution that remains behind. This is best done by treating the watery solution with barium hydroxide solution to the point of neutralization, evaporating to dryness, weighing the residue of barium salts of fatty acids and some barium hydrate in excess, redissolving in water, determining the barium by precipitating it with sulphuric acid as sulphate and subtracting the weight of the barium from that of the above dried residue. The difference in weight indicates the amount of volatile fatty acids present.

LACTIC ACID, PARA.



Paralactic acid, $C_3H_6O_3$, occurs in urine normally only after severe muscular exertion. Pathologically it is found (1) in conditions in which the bodily oxidative processes are interfered

with; (2) whenever there is excessive destruction of glycogen with relatively inadequate oxidation of the latter, or when there is an abnormal amount of destruction of carbohydrates; and (3) whenever muscular catabolism is increased to such a degree that the paralactic acid can not all be converted in the circulation into urea; (4) as ammonium lactate, whenever hepatic functions are so deranged that lactic acid can not be converted into urea. Hence we find it present in cases of severe muscular exertion, violent epileptic fits, trichinosis, experimental ligation of the trachea, carbon-monoxide asphyxiation, acute laryngeal stenosis, long-continued anemic conditions, leukemia, rickets, osteomalacia, pernicious vomiting of pregnancy, and diabetes mellitus. As ammonium lactate it occurs in acute yellow atrophy of the liver, marked cirrhosis, biliary obstruction with diapedesis of bile, and poisoning by phosphorus, strychnine, curare, morphine, amyl-nitrite, veratrine, and arsenic.

In diabetes mellitus lactic acid is apparently one of the agents causing acidosis.

Isolation.—The method of Araki is as follows: the 24 hours' urine is evaporated down until only 50-60 c.c. in volume, treated with 500-600 c.c. of 95 per cent. alcohol, and set aside for 12 hours. It is then filtered and the alcohol distilled off. The fluid remaining is acidified with phosphoric acid and repeatedly extracted with five times its volume of ether. The latter is evaporated until a thick yellow syrup is obtained, which is dissolved in water, filtered, and the filtrate treated with pure lead carbonate in substance, heated on a water-bath for 30 minutes, allowed to cool, and then filtered. The lead is removed by treatment with H_2S, excess of the latter being then driven off on the water-bath. After filtering, the fluid is concentrated on the water-bath to a thick syrup and extracted with ether. The ether is evaporated and the residue boiled for some time with water and excess of zinc carbonate. The mixture is filtered hot, concentrated to small volume, a little alcohol added to it, and set aside in a cool place for the crystals of zinc lactate and paralactate to form. The latter are gathered on a weighed filter, washed with absolute alcohol, dried in the air and weighed. They are then dried at

110° C. (230° F.) to drive off the water of crystallization. If the loss in weight is 13 per cent., then the crystals were those of the paralactate of zinc. This salt is lævorotatory, while the common lactate of zinc is optically inactive.

SUCCINIC ACID.

Succinic acid has occasionally been found in the urine after ingestion of asparagus and asparagin. It is a third acid of the oxalic series. The relationship of the acids of the oxalic series may be seen by the formulas as follows:

Oxalic acid, $H_2C_2O_4$ or COOH.COOH.

Oxaluric acid, $(CON_2H_3)CO.COOH$.

Succinic acid, $COOHC_2H_4.COOH$.

CHAPTER XIV.

PHYSIOLOGICAL CHROMOGENS AND PIGMENTS: UROCHROME, UROERYTHRIN, UROBILIN. THE DIAZO REACTION.

The coloring matters of normal urine and the solubility of them.
Chemistry of urochrome; chemical properties and spectroscopic appearances.
Extraction of urochrome.
Uroerythrin; source; chemistry; significance.
Clinical test for uroerythrin; extraction and spectroscopic appearance.
Action of light, acids, and alkalies upon uroerythrin.
The chromogen of urorosein; action with mineral acids and significance.
Clinical tests: Robin's and the Nencki-Sieber reaction.
Urobilin; chemistry and source.
Physiology of urobilin; origin and quantity.
Clinical significance of urobilin; relation to infections, hepatic diseases, etc.
Clinical notes on urobilin icterus; pernicious anemia.
Significance of Ehrlich's aldehyde reaction for urobilinogen; diseases of the liver, severe myocardial insufficiency, and infections.
Clinical tests: Ehrlich's aldehyde reaction for urobilinogen.
The spectroscopic test for urobilin; the zinc acetate test.
Miscellaneous tests for urobilin; phosphotungstic acid and the biuret reaction; ammoniacal zinc-chloride test, etc., etc.
Ehrlich's diazo reaction; chemical cause of the reaction, and occurrence in diseases.
Formulas for the preparation of the reagent solutions.
Chemistry of the reaction.
Different methods of performing the test.
Significance of the reaction; occurrence in typhoid, measles, tuberculosis.
Relation of the reaction to the prognosis in tuberculosis and cancer.
Drugs whose presence in the urine simulates the reaction.
Arnold's meat reaction.

The coloring matters of normal urine have been named urochrome, uroerythrin, and urobilin. A chromogen also occurs

which yields urorosein. Normal urine also contains a substance which yields a color with Ehrlich's test for typhoid fever (diazo reaction.)

The solubilities of the urinary pigments are as follows:

Soluble in cold water,—urochrome, urorosein.

Soluble in alcohol,—urochrome, urobilin.

Soluble in boiling alcohol,—the above three and uroerythrin.

Soluble in amyl alcohol,—all four, urochrome only sparingly so.

Soluble in acetic ether,—urochrome and uroerythrin, both slightly.

Soluble in acetone,—urochrome slightly.

Soluble in ether,—urobilin.

Soluble in chloroform,—urobilin.

Soluble in dilute acids,—urobilin and urorosein.

Soluble in ammonia,—urobilin.

Urochrome is insoluble in benzole and urorosein in carbon disulphide. Uroerythrin is difficultly soluble in cold water and urobilin is slightly soluble in it.

UROCHROME.

This substance is the principal coloring matter of urine, to which it imparts yellow, orange, and even brown tints. It is a derivative of the normal coloring matter of the blood.

Chemistry.—Urochrome contains nitrogen, but no iron. It also contains more or less urobilin. It may be precipitated by phosphotungstic and phosphomolybdic acids, by acetate of lead, silver nitrate, mercuric acetate, and by saturating with ammonium sulphate. Decomposed by acids it furnishes a brown or black substance, and its acid-alcoholic solution shows spectroscopically a faint narrow absorption band between F and G, its left edge bordering on F.

Extraction.—The urine is acidulated with 0.1-0.2 per cent. sulphuric acid, filtered, and saturated with ammonium sulphate. The resulting precipitate is dried and extracted with warm, slightly ammoniacal alcohol. The alcohol is evaporated and the pigment obtained is an amorphous, reddish-brown substance readily soluble in acidulated water, chloroform, and alcohol, but almost insoluble in ether and benzole.

An alcoholic solution of urochrome, according to Garrod, if treated with aldehyde, gives a rich orange-yellow color, and an absorption band like urobilin. Moreover, a green fluorescence like that of urobilin can be obtained with zinc chloride and ammonia.

UROERYTHRIN (ROSACIC ACID, PURPURIN).

This term is given to the pigment which imparts the salmon-red color to sediments of urates or uric acid. It is a derivative of the normal coloring matter of the blood.

Chemistry.—Uroerythrin contains 62.51 per cent. of carbon and 5.79 per cent. of hydrogen. It is chemically related to hemoglobin, hematoidin, and bilirubin. It exists for the most part in urine in chemical combination with uric acid. It may be extracted from a reddish urate sediment by boiling alcohol.

Significance.—Normally the amount is small, but an increase is noted in acute febrile disorders, especially at the height of the disorder, in extensive diseases of the liver, especially circulatory disturbances, alcoholic cirrhosis, chronic diseases of the heart and lungs, chronic renal hyperemia, hepatic carcinoma, spinal curvature, and in all conditions associated with increased destruction of red blood corpuscles, malaria, etc.

Physiologically an increase follows muscular activity, profuse sweating, and excessive eating.

Clinical Tests.—If the sediment of the urates has a red-rose color, uroerythrin is increased. If there is no such sediment, but if a salmon-red color is imparted to the precipitate when barium chloride or neutral lead acetate solution is added to the urine, uroerythrin is increased.

Extraction.—Shaking the urine gently with amyl alcohol removes the uroerythrin, and the orange solution shows spectroscopically two ill-defined absorption bands, one with its left border midway between D and E, its right border inclosing E and the other with its left border to the right of b and its right border inclosing F. The right band is somewhat darker than the left and the light space between the two is somewhat difficult to see.

On exposure to the light the pigment is bleached. Addition of strong sulphuric acid to its solutions changes it to carmine-red, which on addition of an alkali changes from purple to blue and to green.

UROROSEIN.

In every normal urine, according to Robin, there is a small amount of a chromogen which yields a rose-red color (urorosein) with mineral acids.

Urorosein is increased (1) by vegetable diet and (2) in many diseases: as, tuberculosis, pernicious anemia, marked chlorosis, diabetes mellitus, osteomalacia, appendicitis, nephritis, and especially in diseases of the stomach.

Clinical Tests.—(1) Add to the urine one-tenth its volume of hydrochloric acid and filter. The red stain on the filter is urorosein (Robin). It is also demonstrated in the Jaffé test for indican after the indigo-blue has been extracted by the chloroform, a reddish liquid appearing above.

(2) In order to identify it positively, add 5 to 10 c.c. of 25 per cent. sulphuric acid to 50-100 c.c. of urine. A reddish color appears on standing, which may be extracted with amyl alcohol (Nencki and Sieber's reaction).

UROBILIN (UROPHAËIN HYDROBILIRUBIN).

This is by far the most important of the three urinary pigments last mentioned, and of late has been given much attention by investigators. Urines containing it in considerable amount are dark-yellow or dark brownish-red in color, and their foam is also yellowish or yellowish-brown, thus resembling icteric urines.

Chemistry and Source.—Urobilin, $C_{32}H_{40}N_4O_7$, is present in the urine chiefly as a chromogen, urobilinogen, which on exposure to sunlight gives urobilin. In some diseases there appears to be an increased amount of free urobilin itself in the urine.

Physiology.—The quantity per 24 hours ranges normally from 30 to 120 milligrammes; in diseases as much as 800 milligrammes may be found.

The origin is a matter of dispute and there are several theories. It is probable that a certain amount of it is formed in the intes-

tine as the result of the reducing action of certain bacteria on bile pigments, *(enterogenous formation theory)*. Its presence in the urine may be due to the failure of the liver properly to remove the quantity brought to it by the portal vein.

Urobilinogen is of pyrrhol nature and urinary urobilin is probably identical with the stercobilin of the feces.

Normally the liver is able to excrete the urobilinogen substances derived from the intestine and allows only a trace of them to appear in the urine. Physiologically it has been noticed that the amount is greater in tropical than in temperate climates.

"**Pathologically** when the complete arrest of the biliary circulation arises, urobilin is formed in other parts and, under these conditions, it arises in the tissues themselves. It is due to a transformation of bilirubin, from the bile circulating in the blood into a less toxic body than urobilin or its chromogen. This is not the only origin, because in other circumstances urobilin originates directly from the blood when absorption of an hematic collection has taken place, due to the transformation of hemoglobin. . . .

"Urobilin having a digestive origin should go through the hepatic parenchyma by way of the portal system. If the liver is healthy, it transforms this pigment, but if, on the other hand, it should be diseased, its protective part is nil and it allows the urobilin to pass, from which fact the entire vascular system becomes invaded by this product, after which it is eliminated by the urine. Consequently this urobilinuria is the expression of an hepatic insufficiency. In other cases urobilinuria is merely temporary and only indicates an exaggerated destruction of the blood or biliary pigments. The kidney, as an eliminating gland, also plays a certain part. A lesion of its parenchyma may be accompanied by a simple permeability to chromogen, if the lesion is mild, but if, on the contrary, it is marked, an absolute impermeability will result, so that one will have a urobilinhemia without urobilinuria, from which arises a certain prognostic importance. The absence of urobilinuria in a urobilinogenous affection like pneumonia, and especially the absence of chromogen, should cause one to fear the consequences of renal impermeability, so that the clinical research for this pigment will allow one to judge

the process of globular destruction, as well as to appreciate the functional value of the liver and kidneys." (*Journal of the A. M. A.*)

Clinical Significance.—Urobilin is *increased* in conditions accompanied by destruction of the blood pigments, especially malaria, lead colic, lobar pneumonia, lung infarct, venous thromboses, liver diseases, and certain infections; high fevers, including scarlet fever, also erysipelas, phthisis, acute sepsis, lymphangitis, and acute articular rheumatism; in poisoning by certain drugs: potassium chlorate, pyridin, antipyrin, and antifebrin; in cerebral apoplexy and in hematoma; in severe grades of myocardial insufficiency with edema; in pleurisy with effusion, and especially in exacerbations of pernicious anemia.

The diseases of the liver in which an increase of urobilin is noticed are especially atrophic cirrhosis, carcinoma, and trauma. It is also observed in diseases of the bile passages, after complete obstruction of the bile ducts, and in miliary abscess.

Urobilin is only moderately increased in typhoid fever and not at all in diphtheria.

Urobilin is *decreased* or *absent* in the following conditions: *absent* in the urine of the new-born; *absent* or nearly absent in cases of phosphorus poisoning; *decreased* and sometimes absent in localized extravasations of blood, as in gun-shot wounds or after rupture of aneurism. *Decreased* in diphtheria, localized septic states, severe nephritis, during obstruction of the bile ducts, in severe cases of diarrhœa and severe grades of destruction of the liver substance.

Clinical Notes.—The term *urobilin icterus* is given to the conditions found in both atrophic and hypertrophic forms of hepatic cirrhosis in which the urine contains an increased amount of urobilin.

In *pernicious anemia* urine of red color from urobilin, even when of lowered specific gravity, affords a diagnostic sign of some importance.

Berghausen, of Cincinnati, has investigated the clinical value of Ehrlich's aldehyde reaction for the urobilinogen substances antecedents of the urobilin. (*J. A. M. A., LIV.*, No. 21). His con-

clusions are as follows: "The clinical value of the aldehyde reaction obtained by adding the reagent to cold, freshly passed urine, and then noticing changes in the color, at first in the cold and then on heating, is manifest, though at times limited."

The color reaction in the cold is of pathological significance only when a distinct scarlet color is obtained.

When the reaction persists following free purgation a pathological condition is at hand.

The reaction is most commonly present in diseases of the liver and bile passages, severe grades of myocardial insuffifficiency and certain infectious conditions, as lobar pneumonia and malaria.

The reaction is not a constant one, even in apparently severe grades of the above conditions, presumably because the liver is still efficient in excreting any normal or increased amount of urobilinogen offered it.

Localized infections are more seldom accompanied by this reaction, and when it does persist in such cases the condition of the intestines and liver should be taken into consideration.

In early grades of myocardial insufficiency, of gall stone trouble, and of liver disturbances, the reaction is often a negative one. The appearance of the reaction in such cases, previously negative, would arouse suspicions of disturbances in the hepatic function; inversely the disappearance of a reaction previously positive would indicate improvement.

The positive reaction is not constant in localized extravasations of blood into the tissues.

When the reaction is positive, some care must be exercised in the selection of an anesthetic for operations, since it is well known that chloroform, for instance, can be a direct liver poison.

Absence of the reaction, both in the heat and in the cold, would indicate obstruction in the flow of bile into the intestines.

This condition is also obtained in cases of severe diarrhœa, in the newly-born, and in severe grades of destruction of the liver substance.

(By "this condition" it is evident that he means absence of the reaction. *C. M.*)

Clinical Tests.—Urobilin may be detected in the urine (1) as

urobilinogen substances of pyrrhol nature, and (2) as urobilin itself. It is well to test for both.

(I) To detect the urobilinogen substances *Ehrlich's aldehyde reaction* is used, as follows: 20 grammes of dimethylaminobenzaldehyde, para, are dissolved in 1000 c.c. of dilute hydrochloric acid (made by diluting 150 c.c. of the strong acid to 1 liter with distilled water). To about 5 c.c. of the freshly voided urine cooled to room temperature 5-10 drops of the aldehyde reagent are added, and after shaking allowed to stand a minute or two. Normal urine gives a color varying from yellow to faintly red, but urines containing urobilinogen in pathological amount give a distinctly scarlet reaction in the cold.

(II) Urobilin as such is detected in urine (1) by its absorption spectrum, and (2) by its green fluorescence with zinc salts.

For the spectroscopic test the urine is placed in a test-tube or small trough with glass sides and examined with a small pocket spectroscope (Fig. 18.) A few drops of Lugol's solution

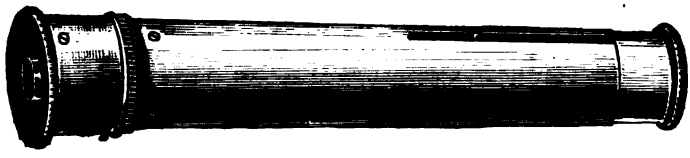


FIG. 18.—Bausch and Lamb Pocket Spectroscope.

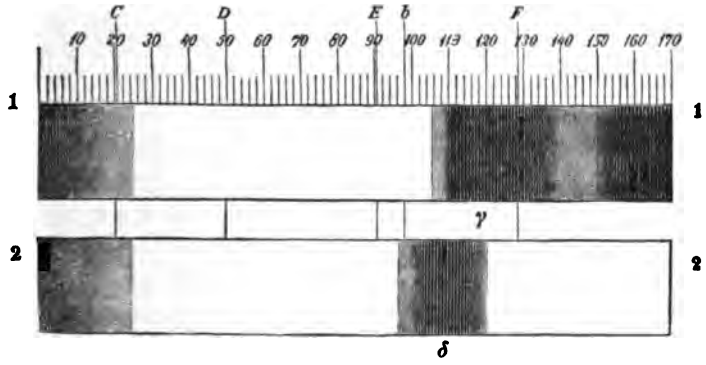
(iodine in potassium iodide solution) should first be added, to convert the urobilinogen into urobilin.

If the urine is deeply colored, it should be diluted. The characteristic absorption-band is between the green and blue parts, between the lines b and F (Fig. 19.)

In many cases the bands are best seen by floating water on the surface of the urine, letting stand an hour and then examining the water above the urine spectroscopically. The test has been modified by adding a little hydrochloric acid to 10 c.c. of urine and shaking *gently* with 5 c.c. of amyl alcohol. Draw off the amyl alcohol extract and examine spectroscopically.

The Zinc Acetate Test.—A test attributed to Schlesinger may be found in some of the German books on clinical diagnosis and

has been copied (unfortunately) into American articles and books. The test is given as one in which equal parts of zinc acetate solution (10 per cent. in alcohol) are mixed with urine and the whole filtered. A green fluorescence in the filtrate shows urobilin. This test is preposterous for the reason that a ten per cent. solution of zinc acetate in alcohol can not be made, if by alcohol is meant absolute alcohol or any alcohol except much diluted. By some writers the test is given as involving a ten



1, Acid urobilin; 2, alkaline urobilin (after Neubauer and Vogel).

FIG. 19.—Acid and Alkaline Urobilin.

per cent. aqueous solution. But the correct performance of the test is given by Dixon Mann, the English writer: make up a solution of zinc acetate as follows: dissolve ten grammes of the acetate in thirty of ammonia water, and to it add 80 grammes of 90 per cent. alcohol and 20 of acetic ether. Filter. To half a test-tube full of urine add one quarter its volume of the solution and filter. The filtrate shows a green fluorescence if urobilin is present.

According to Hawk the zinc acetate test may be used as a ring test, but he does not specify the strength of the alcoholic solution to be used. Hawk's ring test is as follows: acidify 25 c.c. of urine with 2-3 drops concentrated hydrochloric acid; add 5 c.c. of chloroform, and shake the mixture. Draw off the chloroform, place it in a test-tube, and add carefully 3 to 5 c.c.

of an alcoholic solution of zinc acetate. Observe the formation of a green ring at the zone of contact of the two fluids. If the tube is shaken, a fluorescence may be seen.

Miscellaneous Tests.—Phosphotungstic acid precipitates urobilin from urine, and the precipitate dissolved in slightly acidulated water should give, with excess of sodium hydroxide and dilute solution of cupric sulphate, the biuret reaction, *i. e.*, a rose-red color (not reddish-violet or blue). Albumoses should be absent, as they also give this reaction.

Ammoniacal Zinc Chloride Test.—Render some of the urine ammoniacal by the addition of ammonium hydroxide, and after allowing it to stand a short time filter off the precipitate of phosphates and add a few drops of zinc chloride solution to the filtrate. Observe the green fluorescence. Examine the fluid by means of the spectroscope and observe the absorption-band, which occupies much the same position as the absorption-band of urobilin in acid solution.

Gerhardt's Test.—To 20 c.c. of urine add 3 to 5 c.c. of chloroform and shake well. Separate the chloroform extract and add to it a few drops of diluted Lugol's solution. Render the mixture alkaline with dilute solution of potassium hydroxide and note the appearance of a yellowish color. The solution usually exhibits a green fluorescence.

Wirsing's Test.—To 20 c.c. of urine add 3 to 5 c.c. of chloroform and shake gently. Separate the chloroform extract and add to it a drop of an alcoholic solution of zinc chloride. Note the rose-red color and the green fluorescence. If the solution is turbid, it may be rendered clear by the addition of a few c.c. of absolute alcohol.

Ether-Alcohol Test.—Mix urine and pure ether in equal volume and shake gently in a separatory funnel. Separate the ether extract; evaporate it to dryness, and dissolve the residue in 2 or 3 c.c. of absolute alcohol. Note the green fluorescence. Examine the solution spectroscopically and observe the characteristic absorption-band.

Alcohol-Sulphuric Acid Test.—The presence of urobilin can be demonstrated by precipitating 15 c.c. of urine with milk of lime

and extracting the precipitate with 15 c.c. of alcohol acidified with sulphuric acid. Normal urine colors the alcoholic solution faintly red; urobilin in increased quantity colors it up to a brownish-red.

Or, precipitate the urine with a mixture of barium hydroxide and barium chloride. If notable quantities of urobilin are present, the precipitate is thus colored more or less brownish-red. On boiling with the acidulated alcohol the pigment is extracted and imparts a brownish or pomegranate-red color to the alcoholic solution.

EHRLICH'S REACTION (DIAZO REACTION).

Normal urine contains a substance supposedly alloxyproteinic acid, or a similar body which yields a peculiar reaction, as follows: treated with an equal volume of a thoroughly saturated solution of sulphanilic acid in 5 per cent. hydrochloric acid to which an 0.5 per cent. solution of sodium nitrite has been added in the proportion of 1 to 40 or 1 to 100 and the mixture rendered strongly alkaline with ammonia water, a more or less well marked orange color appears. In many diseases, especially typhoid fever, the color varies in intensity from a light carmine to a deep garnet-red.

Chemistry.—The reaction is dependent upon the presence of diazo-benzene-sulphonic acid formed by the reaction between the chemicals with which the aromatics in the urine form anilin colors. Th solutions are made as follows:

Solution 1:

Sulphanilic acid	2 grammes
Hydrochloric acid (1.19)	50 c.c.
Distilled water	1000 c.c.

Solution 2:

Sodium nitrite (not nitrate)	1 gramme
Distilled water	200 c.c.

The reaction in full is as follows: sodium nitrite and hydrochloric acid yield nitrous acid and sodium chloride. Again, nitrous acid and sulphanilic acid yield diazo-benzene-sulphonic acid and water. Lastly, alloxyproteïnic acid plus diazo-benzene-sulphonic acid yields red diazo derivative.

There are a number of methods for performing the test, as follows: (1) Add 50 parts of the sulphanilic acid solution to 1 of the sodium nitrite, and to 10 c.c. of this mixture add 10 c.c. of fresh filtered acid urine. For this purpose a graduated test-tube is useful. Shake well and overlay with ammonia water in excess, using about 3 c.c., allowing the latter to run gently down the tube. At the juncture of the ammonia and urine mixture will be seen a ring of color which in normal urines is deep yellow, orange, or orange-red, but in typhoid fever (and some other diseases) is a carmine-red with a pink or rose-colored foam. When the tube is inverted so that all the contents are mixed the entire liquid becomes crimson and the foam remains pink. Allowed to stand 24 hours a precipitate is formed on the upper surface of which is a zone of dark greenish-black or violet. If the mixture be poured into a white dish containing water, the color of the liquid becomes crimson, in the case of normal urine, yellow.

The only colors of diagnostic importance are the carmine or crimson in the liquid and the pink or rose in the foam.

We are indebted to Dr. Charles E. Simon for careful study of the diazo reaction in his invaluable work on Clinical Diagnosis, which should be read by all interested in the reaction of Ehrlich. Dr. Simon's color reaction is shown in Plate I.

(2) Mix 100 parts of the sulphanilic acid solution with 1 of sodium nitrite, and proceed as above. The reaction will be negative in a number of diseases with this method, hence the writer (following Charles Lyman Greene) prefers the proportion of 100 to 1.

(3) Mix the sodium nitrite and sulphanilic acid solutions in either proportion; add equal parts of urine, shake thoroughly, and quickly add the ammonia without floating it. The test is positive if both fluid and foam are red. Normal urine shows a brown-yellow color. If the red color and foam be present on standing, a precipitate forms, the upper portion of which is colored blue, green, greenish-black or violet.

(4) Some clinicians prefer to add one-tenth volume of ammonia quickly and to shake the whole. Others mix 15 c.c. of



PLATE I.

Ehrlich's Diazo-Reaction, as modified by Simon. The orange color in the lower portion of the test tube may be obtained in any urine; the dark carmine ring indicates the presence of the reaction in a well-pronounced degree; the colorless zone above is intended to indicate the ammonia that has been added. (After C. E. Simon.)

urine with 15 of sulphanilic acid, shake gently, add 5 drops of sodium nitrite solution and then the ammonia, and shake well.

(5) Some authorities advise addition of the ammonia drop by drop.

(6) Dissolve 1 gramme of sulphanilic acid in a mixture of 350 c.c. of water and 15 c.c. of hydrochloric acid. Also make a solution of 0.5 gramme of sodium nitrite in 100 c.c. of water. Five c.c. of urine are mixed with an equal volume of the sulphanilic solution, 3 or 4 drops of the nitrite solution are added and finally 1 or 2 c.c. of ammonia water.

(7) Instead of sulphanilic acid use paramido-acetophenol 50 grammes, hydrochloric acid 50 c.c., water to make 1000 c.c. Add 4 drops of the sodium nitrite solution to 10 c.c. of this mixture, mix well, add 10 c.c. of urine, shake well, and add 3 c.c. of ammonia water all at once. The color of the foam is important and characteristic.

(8) To 1000 c.c. of a saturated solution of sulphanilic acid add 50 c.c. of strong hydrochloric. Mix 2.5 c.c. of urine with 2.5 c.c. of this reagent; add 2 drops of the sodium nitrite solution; shake well, and add 7-10 drops of ammonia water.

(9) Dissolve 0.5 gramme sulphanilic acid in a mixture of 100 c.c. absolute alcohol, 5 c.c. of hydrochloric acid and 5 c.c. of glacial acetic acid. Make also a solution of 0.5 gramme sodium nitrite in 50 c.c. of absolute alcohol. Mix 40-100 parts of first solution with 1 of the second.

To 5 c.c. of urine add 1 c.c. of ammonia water and then the mixture as above, drop by drop. If the test is negative add a few drops of the sodium nitrite.

Laboratory Notes.—Certain precautions in regard to the test are necessary: the two solutions should be kept separate in well stoppered amber-glass bottles and the sodium nitrite should be made fresh every day or two.

The urine should be freshly voided: if for any reason it must be kept several days, ether should be added to it.

In case a negative reaction is obtained in a suspicious case it is well to concentrate the urine to a syrup on the water-bath and test again. If the reaction is still negative, dilute the urine back to its original volume with water and test again.

In cases, also, where the reaction is negative, it is always well to try the test, using one-half the volume of the reagent mixture, instead of equal volumes.

Clinical Significance.—The reaction is most common in typhoid fever, measles, and tuberculosis.

In the proportion of sulphanilic acid 40 to sodium nitrite 1, nearly all febrile urines give a red color of more or less intensity. In the proportion of 100 to 1 in severe forms of typhoid the reaction is positive generally at the end of the first week or ten days, sometimes as early as the fourth or fifth day, and persisting until the fever begins to decline. In this proportion the reaction also occurs in some of the exanthems, in certain cases of advanced malignant disease, late in miliary tuberculosis and in febrile cases associated with septic absorption.

In mild cases of typhoid the reaction is said to be absent, but much care must be taken before pronouncing the test negative, and different methods should be faithfully tried as above before arriving at such a conclusion.

Statements as to its presence in a great number and variety of diseases must not be taken too seriously unless the technique of the test be described. Greene, using dilutions of 100 to 1 and insisting upon the pink or rose colored foam, was able to rule out many conditions in which the reaction was said to be found.

It occurs in tuberculosis and septicemia and in the former is usually, though not invariably, of bad prognostic import. It can not be used to distinguish typhoid from miliary tuberculosis.

It is of value in the early diagnosis of measles, in which it is almost constantly present.

If present in cases of malignant disease, the prognosis is most serious.

In cases of peritonitis, pleurisy, and nephritis, the reaction suggests a tubercular condition.

It is almost never found in acute articular rheumatism and meningitis; it may or may not be found in pneumonia, scarlet fever, diphtheria, erysipelas and tuberculosis, though Greene

insists that in the proportion of 100 to 1 it is absent in pneumonia.

In many cases where the condition is apparantly typhoid the occurrence of the diazo reaction is strong evidence in favor of this fever.

The following drugs cause a positive reaction when administered to the patient: alcohol, chrysarobin, creosote, cresol, dionin, guaiacol, heroin, morphine, naphthalene, opium, phenol, tannic acid and some others.

In alkapton urine a brown ring due to action of the alkali should not be mistaken for the red color due to the diazo reaction.

A Preliminary Stage in Ehrlich's Diazo Reaction in the Urine of the Tuberculous.—According to M. Weisz there are urines which do not yield, when freshly voided, the diazo reaction, but which react positively on standing for some time. This inconstancy of the diazo reaction in advanced pulmonary tuberculosis is due in part to the occurrence of a preliminary stage. The organism of a patient affected with advanced tuberculosis is no longer able to produce the principle of the diazo reaction as such, but he excretes it in the form of a preliminary substance. The latter is converted into the principle giving the typical Ehrlich reaction when kept in the incubator and occasionally even when standing in the cold. The diazo reaction can, therefore, only be considered negative when it does not occur after the urine has been in the incubator for twenty-four hours. (*Archives of Diagnosis.*)

Arnold's Reaction.—Arnold's reaction consists of the production in the urine of a violet coloration when sodium nitro-prussiate has been added, which color changes to purple-red and yellow. While Arnold has observed this change in color only after the ingestion of meat it will also ensue after eating of baked cheese, butter, etc. However, after ingestion of boiled or roasted meat the reaction is so intense that it may be designated as a typical meat reaction. In pathological conditions the reaction was positive in typhoid fever, scarlet, measles, hemorrhagic nephritis, etc. (Archives of Diagnosis.)

CHAPTER XV.

CERTAIN ORGANIC PHYSIOLOGICAL CONSTITUENTS OF URINE.

Carbohydrates in minute quantity:—glucose, isomaltose, animal gum.
Animal gum: constitution; physiology; significance; test.
Isomaltose;—properties, significance, detection.
Chondroitin-sulphuric acid; chemistry and properties.
Mucus and the mucous cloud; composition, physiology.
Determination of the total carbohydrates; method with benzoyl chloride.
Urinary ferments; urinary pepsin, ptyalin, etc.; significance and detection.
Ptomaines and leukomaines; isolation by the method of Baumann and von Udránszky.
Pressor bases in urine: urohypertensine, etc.; relation to gout, etc.

Among other organic normal constituents not yet mentioned certain carbohydrates are found in minute amounts in normal urine, viz., glucose, animal gum, and isomaltose. There are also related bodies, namely, paired glycuronic acid compounds, chondroitin-sulphuric acid, nucleic acid, the mucoid body of the mucous cloud or nubecula, and pentoses. These carbohydrates in all may reach an amount of 2 grammes in 24 hours, measured as glucose. There may be even as much as 1 gramme of glucose normally in the 24 hours' urine.

For the further consideration of glucose see "Abnormal Constituents,—Sugars."

Animal Gum.—This substance is probably not one, but a group of bodies precipitable by alcohol, and of the nature of pentose or identical with dextrine.

Normally it occurs in quantity from 0.1 to 0.2 gramme. It is not fermentable, is slightly dextrorotatory, and gives with the cupric tests a precipitate which does not blacken when boiled.

It is much increased in diabetes mellitus. It is precipitated by Baumann's method with benzoyl chloride as an ester. (See below.)

Isomaltose can be demonstrated as a benzoate in normal urine. It reduces the copper and bismuth solutions, is dextrorotatory, ferments very slowly, and forms an osazone with phenylhydrazine which occurs in very fine crystals, melting at from 150° to 153° C. (302°-307.4° F.). It is precipitated by Baumann's method.

Maltose has been found in the urine in cases of malignant growth and in a case of supposedly pancreatic disease.

Its recognition depends essentially upon the formation of its osazone and the identification of the latter by its melting point (206° C., 402° F.)

It readily ferments and reduces the metallic oxides in alkaline solution, but not so vigorously as does glucose.

Of the bodies related to the carbohydrates the glycuronic acid compounds have already been considered. (See "Aromatics.")

Chondroitin-Sulphuric Acid.—$C_{18}N_{27}NO_{14}.SO_3$ is found in the mucins and is a conjugate sulphate. Boiled with hydrochloric acid it splits into chondroitin and sulphuric acid. It is an amorphous, mucilaginous substance soluble in water. It occurs in the urine in traces.

Nucleic acid has already been considered. (See Chapter VII.)

Mucus.—Normal urine on standing deposits a slight cloud of mucus which is called nubecula. It tends to float in mass in the center of the urine, but sinks when the latter is of low specific gravity. It is much more marked in the urine of women on account of admixture with vaginal mucus, especially in the urine voided on rising. When the nubecula floats in the urine, addition of acetic acid will precipitate it. In catarrhal diseases of the urinary tract the amount of mucus is increased, until the whole urine appears cloudy soon after it is voided, and the cloud sinks more rapidly than in health.

The mucous cloud consists of insoluble mucus, mucous corpuscles and large pavement epithelium, together with a soluble substance in minute amount precipitable by acetic acid.

The total quantity of pure mucus is only a trace, 4.5 grammes in 260 liters of urine. The soluble portion of the mucous cloud yields a reducing body. (See "Albuminuria.")

Pentoses occur in the urine of those in health, especially after drinking beer. Owing to their relation to other sugars they will be discussed in connection with them.

Determination of Carbohydrates.—The total of the carbohydrates in urine may be determined by Baumann's method as follows: the earthy phosphates are removed by treating the urine with soda-lye and allowing the mixture to stand for 24 hours. The precipitate of phosphates is filtered off and the filtrate treated with 4 to 5 c.c. of benzoyl chloride (free from chlorine-benzoyl chloride and benzaldehyde) and 40 c.c. of 10 per cent. soda-lye for every 100 c.c. of the filtrate in order to prevent formation of a sticky mass of benzoic acid esters that cannot be separated by filtration.

The mixture is thoroughly shaken for 15 minutes, or preferably longer, until no odor of benzoyl chloride is perceptible, when it is neutralized with hydrochloric acid and the precipitate allowed to settle at the bottom of the flask. The esters are filtered, dried, and weighed. Normal urine may yield in 24 hours from 2 to 3 grammes of these esters.

Laboratory Note.—Benzoyl chloride, C_6H_5COCl, is a product of distillation of benzoic acid, with phosphorus pentachloride. It is a colorless irritating oil which acts upon hydroxyl compounds, forming benzoic acid esters, (compound ethers or ethereal salts of benzoic acid.)

Urinary Ferments.—Normal urine contains substances which have the power of digesting fibrin in acid solution, of inverting starch to maltose, and of coagulating milk, but there is no proof that these bodies are identical with pepsin, ptyalin, and chymosin. The writer prefers the terms *urinary* pepsin, etc. In certain diseases of the pancreas lipase has been ound in the urine, also in jaundice and perhaps in traces in diabetes mellitus.

According to Ellinger and Scholtz the peptic ferment of the urine is derived principally from the propepsin which has been reabsorbed from the gastric mucosa. Pepsin or propepsin introduced intravenously may be excreted unchanged in the urine. Ingestion of pepsin and subcutaneous injection of pepsin or propepsin produces no increase in urinary ferment. The amount

of ferment is increased when fasting; it declines after meals. The normal fluctuations of the urinary reaction remain without potent influence upon the ferment excretion. In the presence of hyperacidity or hypersecretion in the stomach, the urinary pepsin is mostly augmented in the morning micturitions; in a few cases much ferment is also contained in the midday urine. Occasionally the urine is free from proteolytic ferment, even when pepsin occurs in abundant amounts in the stomach contents. Concurrence of deficient or reduced quantities of gastric pepsin with large amounts of urinary pepsin points to gastric carcinoma.

In conditions of apepsia gastrica according to K. Takeda there will be observed the occurrence of pepsin in the urine. The occurrence of urinary pepsin in the presence of gastric carcinoma seems to stand in a certain relationship to the cancerous changes, because the pepsin excretion by the urine ceases only when the carcinomatous growth has attained considerable size. For the early recognition of gastric cancer, however, the examination of the urine for pepsin is worthless.

The urinary pepsin has been found to be absent from the urine in typhoid fever and the urinary ferments have been reported absent in severe disease of the nervous system, especially in those with convulsions and loss of consciousness.

Detection.—Allow flakes of pure fibrin to stand for several hours in fresh urine. The fibrin absorbs the urinary pepsin and if removed from urine and placed in weak hydrochloric acid in an oven regulated by a thermostat digestion will occur if urinary pepsin is present in the urine.

Ptomaines and Leukomaines.—Ptomaines have been found in normal urine, according to Bouchard, but their presence is strongly denied by various German investigators. In connection with cystinuria, however, certain diamines (putrescin, cadaverin) have been demonstrated.

Isolation.—To demonstrate these diamines there are two methods: (1) that of Löwy and Neuberg in which they are isolated as phenylcyanates and (2) that of Baumann and von Udránszky, which is more commonly employed as follows:

To the 24 hours' urine 25 c.c. of benzoyl chloride and 200 c.c.

of a 10 per cent. solution of sodium hydroxide are added to every 1500 c.c. of urine: phosphates, carbohydrates, and the greater portion of the diamines are precipitated. After filtration the residue is extracted with boiling alcohol, which is filtered and the alcoholic filtrate concentrated on the water-bath. After concentration the solution is poured into 30 times its volume of water and allowed to stand until the benzoylated diamines crystallize out.

The crystals are collected and repeatedly dissolved in alcohol and precipitated with water until free from carbohydrates. They are finally filtered off, dried over sulphuric acid and identified by their melting point and amount of nitrogen. If both diamines are present the crystals lose their water of crystallization at 120° C. (248° F.) and melt at 140° C. (324° F.). To separate them from each other the crystals are dissolved in a little warm alcohol and treated with twenty times as much ether. Benzoylputrescin is precipitated and the cadaverin remains in solution. Crystals of the former melt at 175° C.-176° C. (347° F.), while those of the latter melt between 129° and 130° C. (264° to 286° F.)

A small portion of the diamines remains in the first filtrate. To isolate them, the liquid is acidified with sulphuric acid and extracted with ether. The ethereal extract is evaporated, and the final solution before congealing, placed in as much of a 12 per cent. solution of sodium hydrate as is required for its neutralization. From three or four times as much of the alkali solution is then added. On standing in the cold, sodium benzoylcystine separates out together with the benzolated diamines. The crystals are filtered off and placed in cold water. This dissolves the cystine compounds, while the diamines remain undissolved. They are soluble in warm alcohol, and can be then separated from each other, as described above.

Pressor Bases.—By shaking the urine with charcoal and extracting with suitable solvents certain substances have been obtained to which the term pressor bases has been applied. They are found in diminished quantity when the blood pressure is high, hence a causal relation to this condition has been assigned to them.

According to Bain, normal urine contains two bases which produce a rise in blood pressure. Urohypertensine, the first base, may be obtained as an oxalate from an ethereal extract of the urine, and it is probably identical with isoamylamine, a base which is formed from leucine. The second base remains behind after extraction of the first base with ether, and it may be then obtained by extracting with amylic alcohol the urine which has been made alkaline with sodium carbonate. Its reaction indicates that it is identical or allied to *p*,-hydrophenylethylamine, a base which is formed from tyrosine. Both bases are obtained during the putrefaction of proteins. Their presence in the urine is explainable on the hypothesis that they are formed by putrefactive processes in the alimentary tract, and they are then absorbed and excreted by the urine. In gouty urine the first base is absent. The amount of the second base in gouty urine is smaller than in normal urine. The decrease in the elimination of these bases will possibly explain the rise in arterial blood pressure frequently observed in gouty patients.

CHAPTER XVI.

RATIOS AND COEFFICIENTS.

The coefficients listed.
The coefficients of the total solids: acidity, Bouchard's, etc.
Nitrogen coefficients:—urea-nitrogen, ammonia-nitrogen, etc.
Aromatic coefficients:—Baumann's, Amann's, etc.
The coefficient of acidity:—total acidity divided by total solids.
Bouchard's coefficient:—total urea divided by total solids.
Coefficient of dechlorination:—total NaCl divided by total solids.
Coefficient of demineralization: mineral salts divided by total solids, etc.
Ratio of the mineral acids to the total solids.
Robin's coefficient of demineralization.
Urea-nitrogen ratio:—nitrogen of urea divided by total nitrogen.
Ammonia-nitrogen coefficient:—nitrogen of ammonia divided by total nitrogen.
Clinical method of author with Doremus' instrument and Malfutti's ammonia process.
Uric acid-nitrogen coefficient:—uric acid-nitrogen divided by total.
Uric acid-nitrogen—urea-nitrogen:—uric acid-nitrogen divided by total urea-nitrogen.
Clinical ratio of urea to uric acid by author's method.
The purine-nitrogen coefficient:—purine-nitrogen divided by total nitrogen.
Creatinine-nitrogen coefficient: creatinine-nitrogen divided by total nitrogen.
Amino acid-nitrogen coefficient: nitrogen of amino acids divided by total nitrogen.
Extractive-nitrogen coefficient: nitrogen of extractives divided by total nitrogen.
Phosphoric-nitrogen coefficient:—phosphoric anhydride divided by total nitrogen.
Clinical ratio of urea to phosphoric anhydride.
Sulphuric-nitrogen coefficient: sulphuric acid divided by total nitrogen.
Chlorine-nitrogen coefficient: sodium chloride divided by total nitrogen.
Clinical ratio of urea to sodium chloride.

Coefficients of gastrointestinal autointoxication and of hepatic insufficiency.
Baumann's coefficient: total sulphuric acid divided by ethereal.
Baumann's reversed coefficient: ethereal sulphuric acid divided by total.
Amann's coefficient: ethereal sulphuric acid divided by total nitrogen.
Combe's coefficient: milligrammes of total aromatics per 100 grammes of nitrogen.
Clinical substitute for Combe's coefficient.
Relation of autointoxication to skin diseases.
The coefficient of hepatic functions: neutral sulphur divided by total sulphur.
The recognition of hepatic insufficiency: three methods.
The recognition of organic liver disease: urobilin tests, etc.
Resumé of ratios readily calculated and important clinically.

Having finished consideration of the various physiological constituents, it remains to study their relations to one another and the clinical significance of these relations.

The relation of the various physiological constituents one to another may be expressed by ratios as x:1 or by a fraction of which the quotient of the numerator divided by the denominator represents what is termed the coefficient of its value.

The various coefficients may be listed as follows:

I. **Coefficients of the Total Solids.**—These are:

1. Acidity (ratio of acidity to total solids).
2. Bouchard's (ratio of urea to total solids).
3. Dechlorination (sodium chloride to total solids).
4. Demineralization (a) (chlorides, phosphates, and sulphates to total solids).
5. Demineralization (b) (phosphates and sulphates to total solids).

II. **The Nitrogen Coefficients.**—These are the following:

1. The urea-nitrogen coefficient.
2. The ammonia-nitrogen coefficient.
3. The uric acid-nitrogen coefficient.
4. Urea nitrogen-uric acid nitrogen coefficient.
5. Total purine-nitrogen coefficient.

6. Creatinine-nitrogen coefficient.
7. Extractive-nitrogen coefficient.
8. Amino acid-nitrogen coefficient.
9. Phosphoric-nitrogen (phosphaturic) coefficient.
10. Sulphuric-nitrogen (sulphaturic) coefficient.
11. Chlorine-nitrogen (chloruric) coefficient.

III. The Aromatic Coefficients.—These in connection with the ammonia coefficient constitute the coefficients of intestinal auto-intoxication as follows:

1. Sulphuric acid (total),—sulphuric acid (ethereal), (Baumann's coefficient).

2. Sulphuric acid (ethereal),—sulphuric acid total; (Baumann's coefficient reversed).

3. Sulphuric acid (ethereal)—total nitrogen (Amann's coefficient).

4. Normal aromatic bodies—total nitrogen or urea (Combe's coefficient).

5. Neutral sulphur—total sulphur (coefficient of hepatic functions).

The Coefficient of Acidity.—This may be calculated by dividing the total acidity in terms of hydrochloric acid by the amount of total solids. On a mixed diet this is expressed by a fraction which averages $\frac{1.75}{63} = 0.027$, since the total acidity averages 1.75 grammes of hydrochloric acid and the total solids for a person between 20 and 40 years of age weighing 75 kilogrammes (150 pounds) 63 grammes (975 grains).

☞ This coefficient is far more accurate as an index of urinary acidity than the ordinary clinical determination with decinormal sodium hydroxide alone, which latter fails to take into account the solid content of the urine.

Attention, however, must be paid to the age, weight, and diet of the individual in calculating the total solids, and the various precautions described in Chapter IV. carefully observed.

It is better whenever possible to determine the total solids chemically by evaporation and weighing. Nevertheless, if due regard be paid to the necessary precautions the estimation by the coefficient or Haeser or Long compared with the acidity by the

Folin method, expressed in terms of hydrochloric acid, will give information of a certain value.

The clinical significance of this coefficient relates to "acidemia" and its possible relation to the production of arteriosclerosis. Patients in whom the coefficient is habitually high should be subjected to diet, hygiene, and treatment to reduce the value.

Bouchard's Coefficient.—This is calculated by dividing the total urea in grammes by the total solids. Accurately, this is done by a quantitative analysis of urea, as, for example, by the Moerner-Sjöqvist or the Folin method, Chapter VI., and by determination of the total solids by evaporation and weighing. The coefficient may be expressed by a fraction which averages $\frac{1}{2}$ or 0.50. If expressed in whole numbers the value of Bouchard normal = 50 per 100, *i. e.*, urea is half the total residue.

☞ Clinically we can appreciate marked variations in this coefficient by dividing the grammes of urea in 24 hours (carefully obtained by use of the Doremus ureometer) by the grammes of total solids estimated with the coefficient of Haeser or Long with corrections for diet, weight, age, etc.

The significance of the coefficient relates chiefly to a decrease in the value, which, other things being equal, denotes vitiated metabolism and insufficiency of nutrition. On a vegetarian diet, however, the cofficient may sink to below 40 per 100, hence care must be taken not to assume a pathological bearing of the coefficient without taking into account considerations of diet; an increase in the value is generally indicative of hearty eating, but is also sometimes noticed in chronic nephritis and in acute diseases in which there is tissue waste and chloride retention, as, *e. g.*, in pneumonia, typhoid fever, etc.

The Coefficient of Dechlorination.—This is calculated by dividing the total sodium chloride in grammes by the total solids. The determination of the sodium chloride is preferably made by the indirect method of Volhard or Volhard-Luetke, Chapter VIII. The coefficient is expressed by a fraction which averages $\frac{1}{6}\frac{2}{6}$ or 0.2, since the sodium chloride averages about 12 grammes per 24 hours and the total solids about 60 grammes.

Hence the coefficient may be expressed as normally 20 per

100, but is subject to marked increase if the patient be fond of salt.

☞ Low values are, therefore, clinically more important as in exudative diseases and nephritis. In pneumonia a steady diminution of this value is an unfavorable sign and in nephritis a low value with increasing edema should cause us to diminish the ingestion of salt by restriction of the diet to milk or saltless articles of food for the time being.

Coefficients of Demineralization.—Two such coefficients have been calculated, namely (a) that of the ratio of mineral salts to total solids, and (b) that of the ratio of mineral salts minus chlorides to total solids.

The mineral salts include the chlorides, phosphates, and preformed sulphates; owing to the importance of the mineral content of the living cell and the evolution of the salts in the organism, the coefficients of demineralization are now the object of interesting study. If we reckon the average excretion of chlorides as 12½ grammes, that of the phosphates as 4.25 grammes, and that of the preformed sulphates as 3.50, then the coefficient may be expressed by a fraction averaging $\frac{20}{60}$ or $0.33 = 33$ per 100. Amann gives the value of this coefficient as 30, but on what basis of determination is not stated by him.

Sodium chloride is readily determined as above, but the determination of the phosphates as such, *i. e.*, earthy and alkaline, and of the preformed sulphates, *i. e.*, of sodium and potassium, is somewhat tedious. A more ready method clinically would be to determine the relation of the sum of the acids,—hydrochloric, phosphoric, and sulphuric (of the preformed sulphates) to the total solids. Hydrochloric acid averages 8 grammes, phosphoric acid as P_2O_5 2.25 grammes in 24 hours, and sulphuric acid 2.00, which would give as a fraction $\frac{12.25}{60}$ or about $0.20 = 20$ per 100.

The clinical significance of this ratio deals with circumstances causing exaggerated demineralization of the organism, hence an increase in the value is of significance and occurs quite usually in autointoxication.

Another coefficient (b) of demineralization is known as Robin's coefficient of the demineralization of the protoplasm, and

expresses the ratio between the dechloridated mineral salts and the total solids. Since the sodium chloride is derived from the food, subtraction of it from the total mineral salts leaves a remainder which, to a certain extent, is a measure of cellular destruction, hence the term coefficient of cellular destruction. According to Robin the normal value is from 9 to 10 per 100. If from the sum of 12.25 as above we deduct 8 and divide by 60 we obtain about 0.070 for our clinical calue. A determination of the P_2O_5 and of the H_2SO_4 of the preformed sulphates is all that is necessary for the analysis, which gives us by addition the numerator, while the estimation of the total solids gives the denominator. Clinically an increase in this ratio is of the greater importance since it may indicate a considerable degree of organic cell destruction when the value rises to 15 or above. In cases of autointoxication the value has been found to be above 25 on the basis of 9-10 normal. (0.075 = 7½ per 100.)

The urea-nitrogen ratio requires the most careful chemical analysis for the total nitrogen by the Kjeldahl method and the total urea by the Moerner-Sjöqvist or Folin method. There is no ready clinical method.

The coefficient is expressed by a fraction, the numerator of which is the amount in grammes of nitrogen in the total urea in 24 hours' urine. Hence the term coefficient of the ureic nitrogen is desirable. Normally, the range of value is from 83 to 91, since the per cent. of nitrogen furnished by the urea in 24 hours is within that range. Values much below 83 for the coefficient of ureic nitrogen are usually significant of poor utilization of the proteins in the foods. The liver has much to do with the genesis of urea and clinically the determination of urea may show in many cases hepatic insufficiency, but inasmuch as the question of diet has always been considered, it is more desirable that the coefficient of ureic nitrogen be calculated for the reason that this measures utilization of the proteins in the foods whether by the liver or by other parts of the body. A depression, then, of the value below 83 may be regarded as one of the signs of hepatic insufficiency.

The ammonia-nitrogen coefficient is represented by a fraction,

the numerator of which is the amount of nitrogen in grammes of the total ammonia in the urine per 24 hours and the denominator the total nitrogen of the 24 hours' urine. Normally the ammonia nitrogen is about 5 per cent. of the whole, hence the ammonia coefficient is about 5 per 100. This coefficient has also been termed the ammoniuric coefficient. For the determination of the ammonia the Folin method has been largely used in the recent past, but Harrower, of Chicago, finds the Malfutti process to agree with the Folin method in 8 cases in which the comparison was made. The Malfutti process is comparatively simple and in the author's hands possesses a certain clinical value. (See below.)

A rise in the value of the ammonia coefficient is of interest clinically in that it shows on the one hand profound functional and organic failure of the liver and on the other in the acidosis of diabetes mellitus indicates the struggle of the organism against the fatty acids (diacetic, β-oxybutyric, etc.).

A high value of this coefficient is then of serious import to the surgeon about to administer chloroform, since this anesthetic is a direct poison to the liver.

☞ Fortunately we now possess a ready clinical method for measuring hepatic function and degree of acidosis. The author by use of the Doremus instrument for urea and the Malfutti process for ammonia has calculated a ratio which has proved in a number of cases to be of clinical value. It has been found by thousands of analyses of urea with the Doremus instrument that the results normally obtained range from 20 to 30 grammes in 24 hours. A large number of ammonia determinations by the Malfutti process have shown the range of ammonia to be from 0.3 to 1.2 grammes, just as stated by the older authorities, hence the clinical ratio of urea to ammonia has averaged $\frac{25}{0.75}$ or 33 to 1. It has been found in seven cases of diabetes mellitus with abundance of acetone and diacetic acid that this ratio is greatly diminished. In one case of a child 3 years of age it went as low as 10 to 1 shortly before death.

Surgeons in doubt about administering chloroform should avail themselves of this ready method for determining acidosis and hepatic insufficiency.

The uric acid-nitrogen coefficient is expressed by a fraction, the numerator of which is the amount of nitrogen contained in the total uric acid in the 24 hours' urine and the denominator by the amount of the total nitrogen. The Ludwig-Salkowski or the Folin method may be used for the uric acid determination. Normally, the range of the value of the coefficient is from 1 to 2 per 100, being slightly higher in women than in men.

The uric acid nitrogen-urea nitrogen coefficient is expressed by a fraction, the numerator of which should range from 1 to 2 and the denominator from 83 to 91. It is stated by some authorities to range from 2.5 to 3 per 100.

☞ Clinically a ratio may be readily obtained by the use of the Doremus instrument for urea and the Folin process for uric acid from which a certain amount of information is to be had. From several thousand calculations made by the author it has been found that the ratio of uric acid to urea ranges in health from 1 to 35 upward. As E. E. Smith has observed, the higher ratios are of no clinical interest, but a ratio of uric acid to urea 1 to 30 or lower is likely to be pathological and occur in the condition vaguely termed lithemia with malaise, headache, and insomnia. The author has also observed a lowering of this ratio in gynecological cases, as ovarian tumor. Such cases are apparently due to insufficient oxidation and may be taken to indicate in a general way the cry of the organism for more oxygen.

The total purine-nitrogen coefficient is expressed by a fraction, the numerator of which denotes in grammes the nitrogen of the total purine bodies, *i. e.*, uric acid plus purine bases, and the denominator the total nitrogen per 24 hours in the urine. The value of this coefficient is usually given as 2 per 100. The determination of the purine bases is made by the method of Salkowski and the nitrogen calculated added to that of the uric acid to obtain the total purine-nitrogen.

The value rises considerably above 2 in cases of enteritis, owing to phagocytic reaction against microbic invasion, accompanied by a considerable destruction of white cells. It is said that the value falls in autointoxication.

The creatinine-nitrogen coefficient is expressed by a fraction,

the numerator of which is the nitrogen of the total creatinine and the denominator the total nitrogen. Normally, the value is said to be between 4 and 5, and it may perhaps indicate the utilization of nitrogen by the body since some good authorities claim that it contains all the nitrogen really used by the body. The creatinine has in the past been determined by the method of Neubauer-Salkowski of weighing the creatinine-zinc chloride crystals, but the more recent colorimetric method of Folin may make it a clinical possibility to utilize the Doremus urea determination for comparison in a rough way.

The next coefficient of special interest is the **coefficient of undetermined nitrogen,** or the amino acid-nitrogen coefficient. The amino acid-nitrogen coefficient is represented by a fraction, the numerator of which is the nitrogen of the total amino acids and the denominator the total nitrogen. This is obtained by subtracting from the total nitrogen the sum of the various nitrogenous constituents already considered. The value, according to Von Jaksch, ranges from 1.52 per 100 to 3.61. It is increased in hepatic diseases, in typhoid fever, and in some cases of Graves' disease.

It is possible that the toxemias of pregnancy are dependent upon failure of the organism to split up the amino acids, hence the study of this coefficient in such cases becomes of considerable interest.

According to Ewing and Wolf there are three conditions of such toxemia; one characterized chiefly by vomiting, in which we find a low urea-nitrogen coefficient, a high ammonia-nitrogen coefficient, and a high amino acid-nitrogen coefficient; another a pre-eclamptic state in which we find low urea, variable ammonia, and high amino acid; and a third, eclampsia, in which we find urea low in proportion to severity, ammonia variable, and amino acid high, but not so accurately in proportion as urea is low.

The extractive-nitrogen coefficient is expressed by a fraction of which the numerator denotes the amount of nitrogen furnished by the normal nitrogenous constituents not urea, ammonia, nor purine bodies, and the denominator the total nitrogen, hence the

extractive nitrogen includes creatinine-nitrogen, hippuric acid-nitrogen, amino acid-nitrogen, etc. Normally its value is said to be 10 per 100. The methods of determining the various constituents are, with the exception of creatinine, not clinical, and the significance of the coefficient is not clear. It is, however, said to indicate abnormal nitrogenous waste, hence is increased in autointoxication, as a result of interference with digestion and nutrition.

The phosphoric-nitrogen coefficient or phosphaturic, as it is also called, is expressed by a fraction, the numerator of which is denoted by the number of grammes of phosphoric anhydride (P_2O_5) in 24 hours and the denominator by the total nitrogen. The phosphoric acid is determined by the uranium nitrate method. The value of the coefficient ranges from 17 per 100 up to 20. Some authorities state it to be 21.7-23.6. It is lowered in a majority of the cases of autointoxication due to nutritive defects in the phosphoric elements of the organism. It is also lowered in anemia and in all cerebral excitations, especially before or during an attack of epilepsy, in chronic brain affections, except tumors, delirium tremens and acute hydrocephalus. It is low in the progressive paralysis following syphilis, but rises greatly after administration of the iodides. It is low in the excitement stage of mania. The value may rise to as high as 30 or more in apoplexy, brain tumors, tabes, and arthritis deformans, and is high in pernicious anemia.

☞ Clinically we can avail ourselves of the ratio obtained by comparing the amount of urea obtained with Doremus' instrument with that of the P_2O_5 by the method with uranium nitrate. Normally the ratio of P_2O_5 to urea as thus obtained varies from 1 to 8 up to 1 to 12, or urea is to phosphoric acid on an average as 10:1.

The ratio of urea to P_2O_5 is much increased in cases of nervous exhaustion, in chronic nephritis, and especially in Addison's disease (as high as 20 to 1 or over, without increase of urea).

The sulphuric-nitrogen coefficient, also called the sulphuric and the chloruric, is expressed by a fraction, the numerator of which is the total sulphuric acid per 24 hours and the denominator the

total nitrogen. The value ranges from 16.3 up to 18.7 normally. The sulphuric acid is determined by weighing the calcined barium sulphate precipitate formed by adding to 50 c.c. of urine 5 c.c. of strong hydrochloric acid and 10 c.c. of a 3 per cent. barium chloride solution after heating over the flame for 15 minutes.

There is no ready clinical method for comparing the sulphuric acid with the nitrogen. The value is lowered as a rule in cases of autointoxication.

The chlorine-nitrogen coefficient, also called the chlorine and the chloruric, is expressed by a fraction the numerator of which is the total sodium chloride and the denominator the total nitrogen. The value is differently stated owing to the difference in the habits of the persons with reference to eating salt, but it ranges from 70 up to 128 per 100 of nitrogen. The various authorities seem unable to draw definite conclusions from observation of this ratio in chronic diseases, but the author by use of the Doremus instrument for urea in comparison with results obtained from determinations of the sodium chloride by the Volhard-Luetke method has shown the following:

☞ The ratio of urea (hypobromite method) to sodium chloride is normally about 2 to 1. High ratios should suggest acute infectious diseases (especially with exudations) in which the chlorides are retained and serious nephritic conditions with edema in which the kidneys are either impermeable to the chlorides or else the chlorides are retained in the tissues. High ratios are also suggestive of cancer of the stomach rather than simple dilatation, indicating that much tissue protein is being destroyed. A decrease in the ratio of urea to sodium chloride is in general a favorable sign, especially in diseases of the stomach and in acute exudative diseases (pneumonia, pericarditis).

Coefficients of Gastrointestinal Autointoxication and of Hepatic Insufficiency.—Among these is the ammonia coefficient already considered. Formerly Baumann's coefficient and reversed coefficient were deemed essential for the diagnosis of gastrointestinal autointoxication. These coefficients represent the ratio of the total sulphates to the ethereal sulphates or the total sulphuric acid to the sulphuric acid of the ethereal sulphates. But recently

they have been relegated to second place in comparison with Amann's and Combe's coefficient.

Baumann's Coefficient.—This is expressed by a fraction, the numerator of which is the total sulphuric acid and the denominator the sulphuric acid of the ethereal sulphates. Since this figure on division yields always a whole number and the greater the denominator the less the coefficient, a smaller coefficient indicates a greater amount of ethereal sulphates, *i. e.*, a greater degree of intestinal putrefaction, and is, therefore, misleading in a certain way, hence the reversal of it as below.

Baumann's Reversed Coefficient.—This is obtained by inverting the fraction above described so that the total sulphuric acid becomes the denominator. The value is normally 10 per 100, *i. e.*, the sulphuric acid of the ethereal sulphates is one-tenth that of the total sulphuric acid. This coefficient rises in autointoxication, but it is not deemed so reliable as the others, namely, the ammonia-nitrogen and the coefficients of Amann and of Combé. There is no ready clinical method of determining the constituents from which this coefficient may be calculated. The total sulphuric acid is determined as above described by calcining the barium sulphate precipitate and weighing, while the sulphuric acid of the preformed sulphates is obtained by acidulating 50 c.c. of urine with 5 c.c. of acetic acid and addition of barium chloride as before with slight heating. The precipitate of barium sulphate is collected and calcined as before and the sulphuric acid calculated from it. Subtraction of the quantity of acid last found from that of the total gives the sulphuric acid of the ethereal sulphates. Or the amount of calcined barium sulphate found in this second process subtracted from the amount obtained as described above gives a remainder which, multiplied by the constant factor 6.8692, represents in milligrammes the quantity of sulphuric acid of the sulphoethers in one liter of urine.

Amann's Coefficient.—A better measure of intestinal auto-intoxication is the coefficient which is expressed as a fraction with the numerator the sulphuric acid of the ethereal sulphates, and the denominator the total nitrogen. The numerator is, therefore,

derived solely from the putrefaction of the nitrogenous foods and the denominator the total nitrogen derived almost exclusively from the alimentary proteins. Normally, the value ranges from 1.4 to 1.5; in vegetarians perhaps higher, 1.8 to 1.9 per 100 of nitrogen. It may rise above 5 in autointoxication.

Combe's Coefficient.—This coefficient indicates the number of milligrammes of aromatic (ethereal sulphates and oxyacids) per 100 grammes of total nitrogen of urea. Normally, Combe found 200 to 250 milligrammes of aromatics per 100 grammes of nitrogen. In autointoxication the value may rise enormously. In one case of chronic invagination Combe found the value 4250.

There is no ready clinical method for determining the constituents of the numerator, but the ethereal sulphates (indoxyl, etc.) and oxyacids may be determined by colorimetric processes, using Amann's chromometer with results sufficiently exact for clinical purposes.

☞ Clinically, if we can obtain a brilliant blue with our tests for indican, while at the same time the amount of urea with the Doremus ureometer is not above 20 grammes per 24 hours, we may assume a high value for Combe's coefficient.

It has been found that the aromatic coefficients are always greatly increased in autointoxication and the value of calculation of them has been recently shown by modern papers on dermatology. Crocker, Bulkley, Judassohn, and Brocq, assert that a large number of diseases of the skin are caused by the cutaneous elimination of toxic substances, especially prurigo, strophulus, acne, urticaria and certain eczemas. In some cases the autointoxication is of intestinal origin and in some cases not. Examination of the urine by modern methods will usually reveal the source, if intestinal.

The Coefficient of Hepatic Functions.—This term has been applied to the fraction obtained by dividing the neutral sulphur in the urine by the total sulphur.

Normally, the value is from about 13 (in men) to 18 (in women) of the neutral sulphur per 100 of total sulphur.

Since the large proportion of the neutral sulphur of the urine

is derived from taurocholic acid in disorders of the liver (accompanied by biliary stasis and diapedesis of bile), the value of this coefficient rises.

There is no ready clinical method for determining the constituents from which this coefficient is calculated.

The recognition of hepatic insufficiency is brought about, first, by observation of a lowering of the urea-nitrogen coefficient, second, by a rise in the ammonia-nitrogen coefficient and purine-nitrogen coefficient, and third, by the appearance of leucine in the urine, recognized by chemical and microscopical tests.

The recognition of organic disease of the liver is helped by the detection first of the pyrrhol-like urobilinogen substances, and second, by the tests for urobilin itself. (See Chapter XIV.)

☞ **Clinical Resumé.**—For clinical purposes the following may be substituted for the more elaborate processes of the laboratory:

1. The determination of ammonia by the process of Malfutti and the calculation of the ratio of ammonia to urea determined by the Doremus method, values much lower than 20 to 1 (urea to ammonia) indicating a relative excess of ammonia, as in severe cases of diabetes mellitus and in hepatic insufficiency.

2. Calculation of the coefficient of acidity by division of the estimated solids by the acidity determined by the decinormal sodium hydroxide and calculated as hydrochloric acid, the average normal being 0.027, a marked increase showing acidemia and possibly an early stage of arteriosclerosis.

3. Determination of uric acid by the Folin method and of urea by the Doremus with calculation of the ratio, values less than 35 to 1 (urea to uric acid), indicating excess of uric acid as in "lithemia," insufficient oxidation, etc.

4. Determination of urea by the Doremus method and comparison with the results of the indican test of Askenstedt or of the writer: low urea with high indican showing intestinal autointoxication, pus absorption, etc.

5. Recognition of organic disease of the liver by Ehrlich's benzaldehyde test of the freshly voided acid urine for urobilinogen, or the zinc acetate test for urobilin.

CHAPTER XVII.

PATHOLOGICAL CONSTITUENTS IN SOLUTION IN URINE: PROTEINS.

Proteins occurring in urine: albumin, globulin, etc.
Albuminuria, false and true.
False albuminuria: due to pus, etc.
Mixed albuminuria: due partly to pus, etc., partly to renal changes.
Clinical notes on false albuminuria.
True albuminuria: non-nephritic and nephritic.
Non-nephritic albuminuria:—functional, essential, circulatory, hemic, nervous, toxemic, traumatic, dyspeptic, etc.
The albuminuria of nephritis.
Author's clinical chart of nephritis: acute, subacute, chronic, arterio-sclerotic, syphilitic, amyloid, diabetic, etc.
The detection of albumin in urine: collection and preparation of the specimen, filtration, choice of tests.
Five clinical tests for albumin:—Ultzmann's, as employed by the author, Purdy's, the two nitric acid tests, the ferrocyanic test.
Miscellaneous tests: Spiegler's, Tanret's, Roberts', Johnson's, etc., etc.

The various pathological constituents occurring in solution include numerous proteins as below.

PROTEINS.

The proteins occurring in urine are serum-albumin, serum-globulin, the proteoses (albumoses and peptones), nucleoalbumin, the protein or proteins of mucus (mucoid, nucleoprotein, euglobulin), nucleohiston, fibrin and hemoglobin. Egg-albumin may also occur after a diet rich in eggs. The proteins, with the exception of hemoglobin, are colloid and, hence, do not penetrate such animal membranes as are intact.

ALBUMINURIA.

Albuminuria is either false or true, *i. e.*, either due to accidental presence of pus, blood, etc., or else to the renal changes mentioned below.

False albuminuria, also called accidental or adventitious, is commonly found in suppurative diseases of the urinary or genito-urinary tract due to the presence of pus; hence in urethritis, prostatitis, and various abscesses discharging into or draining through the tract. Even in women with leucorrhea, sufficient purulent fluid may drain into the urine, especially into that first voided on rising in the morning, to cause presence of albumin in the urine. Seminal discharges and presence of prostatic fluid may also be responsible for traces of albumin. When the albumin is due wholly to pus in the urine, the quantity is likely to be small and when precipitated to be less white and more flocculent than in true albuminuria. Such albumin does not always settle when sedimented at 1000 revolutions per minute in the centrifugal tubes after precipitation with acetic acid and potassium ferrocyanide. Albumin due to blood in the urine is usually more abundant in quantity than that due to pus or other discharges, and is slightly reddish or brownish in color when precipitated. Albumin in the urine of women due to the presence of a great mass of vaginal epithelium shows more plainly with the heat and acetic acid test than with the salt test. (See below.)

Mixed albuminuria occurs when the albumin is due partly to pus or blood and partly to renal changes, etc.

Microscopic examination of the sediment is usually necessary for determining the kind and significance of the albuminuria present, but a large amount of albumin, dense and white when precipitated, is due to true albuminuria. The converse, however, is not true, since the merest trace of albumin may also be due to true albuminuria, as in chronic interstitial nephritis.

☞ **Clinical Notes.**—1. The presence of albumin in the urine does not in itself signify Bright's disease, and the commonest illustration of the truth of this remark is to be found in cases of severe leucorrhea or gonorrhea.

2. In all cases in which albumin is found in urine the first clinical question to be asked is whether it is false albuminuria or true.

3. The presence of even a trace of albumin is of clinical importance as will be explained further on.

4. In calculous, tubercular, post-gonorrheal and malignant diseases of the urinary tract false albuminuria is clinically common. False albuminuria is also common in the case of elderly men with prostatic and bladder diseases.

Serum Albumin and Globulin.—These substances occur together in urine and the presence of them in readily demonstrable amount is probably always pathological, although in some cases lesions in the kidney can not be proved post mortem. The term *albuminuria* is used to define the presence of these bodies in sufficient amount to be detected by certain clinical tests, especially that of Heller. Albuminuria (true) is due either (1) to changes in the blood which render the proteins more diffusible through animal membranes, (2) to changes in the blood pressure, or (3) to changes in the renal epithelium.

True Albuminuria.—In most cases this condition means changes in the epithelium of the glomeruli or of the tubuli uriniferi in the kidneys. Delayed circulation in the glomeruli or a hydremic state of the blood may also be factors in the production of it. True albuminuria is usually accompanied by the presence of renal epithelium and tube-casts in the urine, but these may be small in amount or even absent at times in some cases of true albuminuria, and most of the time in the condition known as functional and essential albuminuria. (See below.)

Classification of True Albuminuria.—True albuminuria is either (a) non-nephritic or (b) nephritic.

Non-Nephritic Albuminuria.—Albuminuria without the clinical features of nephritis may be classified as follows: (1) functional, (2) essential, (3) circulatory, (4) hemic, (5) nervous, (6) toxemic, (7) traumatic.

Functional Albuminuria.—This term is given to non-nephritic albuminuria occurring under special conditions, and is *transitory*. The causes of it are excessive indulgence in protein foods, severe muscular exertion, especially in those unaccustomed to it, mental shocks, strains, or vasomotor disturbances, as prolonged cold baths, electric shocks, and sexual excesses. Women are subject to such albuminuria just after labor. It occurs not uncommonly

in new-born children during the first fortnight. It is said that any person can, by unduly excessive muscular exertion, bring on functional albuminuria.

☞ **Essential Albuminuria.**—This condition is important clinically only because of the mistakes in diagnosis which it may occasion. The term is used to designate an albuminuria in which the only observed condition is the albumin in the urine. The author has had a large experience with such cases occurring in medical students who discover albumin in their urine while working in the chemical laboratory and are more or less perturbed by the discovery. The condition is almost unknown to the general practitioner, but the life insurance examiner is familiar with it.

The cases occur in young people about the age of puberty and are either the result of abnormal conditions of the circulation during the age of sexual activity or else to an inequality between the development of the kidneys and the rest of the body. The subjects are likely to be anemic or neurasthenic.

The amount of albumin may be considerable (10 to 20 per cent. bulk), but casts are scarce or absent, more likely the latter. It is held, however, by some authorities that the presence of hyaline and even granular casts has been observed in some cases. The author has never found waxy casts nor long dark granular casts in this condition. Essential albuminuria may be *cyclic, i. e.*, occurring in cycles or waves, at certain hours in the day, as, for example, about noon, and again in the evening, or *orthostatic* (postural), *i. e.*, only when the patient is on his feet. In an experience of thirty years, during which about one hundred such cases have been seen by the author, not one, so far as known, has developed into nephritis. The rule is that, as the patient grows older the albuminuria disappears. There are no cardiovascular changes, no ocular changes, and no increase in the blood pressure is noticed in these cases.

According to Langstein an albuminuria in childhood may be considered innocent when an entire year's observation proves the following: (a) that the night urine is always free from albumin,

(b) that there are fluctuations in the albumin-content of the day urine, and (c) that the greater portion of the albumin is precipitable by acetic acid in the cold. (See Mucoid and so-called Nucleoalbumin.) According to Turettini most cases of orthostatic albuminuria are due to lordosis of the lumbar spine. He claims, however, that orthostatic albuminuria may, in some cases, be due to latent organic disease of the kidneys.

Clinical Note.—The author contends that the term orthostatic albuminuria should be restricted to those cases which are strictly essential so far as our diagnostic measures can determine, since in some cases of chronic interstitial nephritis albuminuria of orthostatic type may be noticed.

Circulatory Albuminuria.—In this condition known disturbances of the circulation are present, *e. g.*, (1) *acute renal hyperemia,* as in shock from surgical operations, after use of anæsthetics, after violent exertion (foot ball games), in painful paroxysmal affections (colic, etc.), or (2) *chronic renal hyperemia,* passive congestion, venous stasis in the kidney, as in valvular diseases of the heart or other obstructions of the circulation and pressure on the inferior vena cava from tumors, in pregnancy, etc.

☞ Clinically, this kind of albuminuria is seen most often in the acute form after surgical operations on the genitourinary or urinary tract, and in the chronic form as a result of valvular disease of the heart. Blood may be present in the acute form. In both forms the quantity of urine is decreased and in the acute form may be even suppressed. Tube-casts may be found, but not waxy ones, nor large dark granular. The chronic form in time merges into a nephritic condition (hypostatic contracted kidney). The amount of albumin may be large for a short time in the acute form, but is seldom above 20 per cent. bulk—and usually less—in the chronic form.

Hemic Albuminuria.—In diseases of the blood, such as pernicious anemia or severe anemia, leukemia, purpura, scurvy, jaundice (cholemia), also in diabetes mellitus and other wasting diseases, albuminuria occurs, without true nephritis. A few

tube-casts may also be found in the urine and in jaundice great numbers of granular casts may be found stained with the bile-pigment. The amount of albumin is small in all these cases,—less than 10 per cent. by bulk. In scurvy, hemoglobinuria may increase the amount of albumin.

Nervous Albuminuria.—This term is faulty and is only used because of its brevity, to designate the albuminuria observed in cases of epilepsy, tetanus, migraine, insanity, melancholia, psychoses, delirium tremens, paralyses, etc. The amount of albumin is small and casts are few.

Guillam and Vincent found an enormous amount of albumin in the urine of a woman who suffered from meningeal hemorrhage.

☞ **Toxemic Albuminuria.**—This is an important condition clinically, since it is likely to be mistaken for nephritis by the practitioner. In many infectious diseases albuminuria of short duration due to acute parenchymatous degeneration of the kidneys is observed. The condition is not necessarily found at the time of the highest bodily temperature, but is likely to follow the latter, *i. e.,* is present at the time when the patient suffers most from the toxemia of the infectious disease in question.

☞ Formerly, the term *febrile albuminuria* was used to designate this form of albuminuria. Casts are not likely to be present in number or variety. The condition is temporary, but if it persist after the toxemic symptoms subside and if casts appear, nephritis is developing.

An albuminuria of this kind may be observed early in the course of syphilis. It is possible that severe cases of gastro-intestinal toxemia with indicanuria may be accompanied by albuminuria.

Some writers, *e. g.,* Croftan, insist upon a *dyspeptic albuminuria:* seen in dilatation of the stomach and in many intestinal disorders, accompanied by great indicanuria and finally resulting in nephritis. Ingestion of meat, eggs, milk, etc., may be followed in healthy subjects by transitory albuminuria, and it is this fact that has led Dr. Croftan to study the influence of protein diet

and digestive derangements upon the appearance of albumin in the urine. He has observed this peculiar type of alimentary albuminuria in the following five conditions, viz., motor insufficiency of the stomach, intestinal indigestion, especially with diarrhæa, hepatic insufficiency of advanced degree, rectal feeding, and excessive ingestion of albuminous pabulum.

According to Morse and Crothers albuminuria is found in from 8 to 10 per cent. of infants ill with various diseases of the gastrointestinal tract, but the condition is not a nephritic one and does not commonly result in nephritis, though pyelitis or pyelonephritis is not uncommon.

Albuminuria follows the administration of **irritant and corrosive poisons** in toxic doses, and also that of certain balsams. Drugs likely to cause albuminuria are alcohol, arsenic, antimony, acids (mineral), cantharides, carbolic acid, lead, mercury, phosphorus, sandalwood oil, salicylic acid, silver, uranium, etc., and it has been claimed that an albuminuria occurring with oxaluria, *i. e.*, copious deposit of oxalate crystals, is not nephritic.

Persistent albuminuria following a toxemia, the so-called **minimal albuminuria** of the French, is sometimes noticed. The amount of albumin is small and may be no more than a trace. It is claimed that in some of these cases renal tuberculosis, gout or nephritis follow, while in others the albuminuria alone lasts for years without other conditions developing.

☞ An albuminuria of sudden onset and sometimes great intensity may occur during pregnancy and be followed by the so-called **puerperal eclampsia.** The quantity of albumin is very large, but casts are few or absent and blood may be absent. The quantity of urine is diminished or suppressed and the specific gravity may be high. The condition may subside quickly, but a trace of albumin may remain after recovery, like the minimal albuminuria above mentioned.

Traumatic and Lordotic Albuminuria.—Following accidents and injuries of the kidneys albumin may appear in the urine, but usually the albuminuria is false, due to the pus or blood, or to a real acute nephritis of the hemorrhagic variety, the latter

followed later by a contraction of the kidney (chronic interstitial nephritis) in serious cases.

Changes in the position of the spine appear to cause albuminuria. If the spine is lordotic, albumin may be found, and this disappears from the urine when the lordosis has been corrected. According to Turretini lordosis of the lumbar spine is the most important etiological factor in most cases of orthostatic albuminuria.

Albuminuria may be induced, it is claimed, by faradism of the lumbar region.

THE ALBUMINURIA OF NEPHRITIS.

☞ When nephritis is present, the clinical history, the presence of edema, increase of blood pressure, the changes in the heart and arteries, together with the kind and variety of tube-casts found in the urine, may serve to differentiate the albuminuria from the conditions already considered.

☞ Circumstances pointing strongly to the existence of **acute nephritis** are: (1) history of a previous infection, (2) sudden onset, (3) presence of edema, (4) plentiful presence of albumin and blood in the urine, (5) numerous tube-casts (hyaline, epithelial, blood, leukocyte, yellow granular, reddish granular) in the sediment.

☞ Circumstances pointing strongly to the existence of **subacute nephritis** are: (1) obscure origin or else history of previous infection or of acute nephritis, (2) gradual onset and slow course, (3) presence of edema, secondary anemia, and debility, (4) plentiful presence of albumin and casts in the urine, the latter coarsely granular, fatty and waxy, or else in hemorrhagic nephritis, blood and large dark-reddish casts (hemoglobin casts).

☞ Circumstances pointing strongly to **chronic nephritis** are: (1) imperceptible onset with no previous history or else with a history of infection years before, (2) increased blood pressure, (3) edema seldom a feature, except during acute exacerbations or late in the course of the disease, (4) changes in the heart and arteries (hypertrophy, dilatation, arteriosclerosis,

accentuation of the aortic second sound), (5) acute uremic attacks (dizziness, unconsciousness, coma), (6) nocturnal polyuria, (7) slight albuminuria with but few casts in the urine.

(Clinical chart gratis mailed by author on request.)

DETECTION OF ALBUMIN IN URINE.

FIVE CLINICAL TESTS COMMONLY EMPLOYED.

In order to detect albumin in the urine much depends upon the condition of the specimen examined.

☞ **Collection and Preparation of the Specimen.**—Whenever possible, in addition to the 24 hours' collection, a freshly voided sample of urine should also be provided. When urine is sent from a distance collect in a clean glass vessel, as a pitcher or fruit jar, pour into a clean bottle, add five grains of boric acid of the highest purity to every four ounces of urine, shake well, cork tightly with a clean cork, pack in sawdust and send by express with a letter by mail giving particulars. In cases where only traces of albumin are suspected, select that specimen which is voided during the evening, since traces of albumin are sometimes absent in the morning. Urine containing bacteria is unfit for examination for traces of albumin, since bacterial growths give an albuminous reaction with certain tests. Women should tampon the vagina or use douches before urinating for the purpose of an albumin test, and in all cases should observe strict cleanliness. The habit of furnishing for examination the urine voided upon rising in the morning is not to be commended, since rest is conducive to diminution of the amount of albumin; even in serious cases of chronic nephritis, albumin is sometimes absent in the urine voided on rising. It is often necessary to examine the freshly voided urine of every urination for the 24 hours.

The sample must be clear, for the detection of traces of albumin. In nearly all cases filtration through three thicknesses of Sargent's No. 500 white filter paper is sufficient to clarify the urine. If this is not enough, the filtered urine may be additionally filtered through powdered asbestos or an asbestos fil-

ter, or through fine white sand which has been heated in a crucible to destroy all organic matter. In some cases when the urine contains bacteria filtering through six papers folded together is all that is necessary to clarify it, and this method is recommended by the author when there is enough urine supplied.

Choice of Tests.—Much difference of opinion exists as to the choice of tests. The author has used Ultzmann's test (heat and acetic acid) for many years and prefers it for certain purposes. F. Engels, in a critical review of the commonly employed tests *(Deutsche med. Wochenschrift, Nov. 25, 1909)*, recommends this test as one of the most trustworthy and convenient. All tests in which acids are added *prior* to boiling should be discarded.

I. PRELIMINARY TEST: THE AUTHOR'S METHOD OF EMPLOYING ULTZMANN'S TEST.

☞ 1. Provide a tall, narrow test-tube, holding 10 c.c. and filter the freshly voided or otherwise clear urine into it until three-fourths full. Wipe off the outside of the tube with a clean cloth, holding the tube up to the light so as to see that both tube and urine are entirely transparent.

2. *Boil the upper stratum of the urine over an alcohol lamp flame.* This is done by holding the tube *by the closed end* with the thumb and forefinger of the right hand, inclining it over the flame in such a way that the latter heats the urine about half an inch from the surface of the liquid. Use an alcohol flame so as not to crack the tube by too great heat. Do not at any time let the flame touch the tube above the surface of the liquid. Nearly all beginners fail to observe this precaution, with result that the tube is decapitated, so to speak, loses the upper quarter of the tube as neatly as if chopped off. Boil *thoroughly* thirty seconds, removing from flame whenever the urine threatens to boil over, but do not boil the lower half of the urine at all.

3. Add, drop by drop, boiling after each drop, three to six drops of fifty per cent. acetic acid to the boiling urine. Shake to and fro gently, until acid and upper stratum of urine have thoroughly mixed, then boil again for, say, thirty seconds.

4. Hold the tube against a dark background, as the coat sleeve, or better still, hold it below a window sill of a north window or any window where there is no direct sunlight.

RESULTS:

A. If albumin is present, the upper, heated, acidulated quarter, or possibly third, is now distinctly turbid as compared with the lower, cool, remaining portion of the urine. If ***much*** albumin is present, the whole upper third or half of the urine is milky and flocks soon begin to fall. If only a ***moderate*** amount of albumin is present, the upper quarter or third is cloudy. If there is a distinct turbidity which cannot be seen when the tube is held *up* to the light, we call it a *plain trace*. If there is an indistinct turbidity of the same character, requiring careful adjustment of eye and background, in order to see it, we call it a *trace*. If the turbidity is faint and only seen with great difficulty we call it a *faint* or *doubtful* trace. Such faint traces may be possibly due to proteins of mucus and not albumin at all. They may be observed in the urine of nearly all women.

B. If there is no cloudiness, albumin is absent, but albumoses may be present and undetected.

C. If there was a cloudiness observed on boiling, which becomes clear or nearly clear on addition of acid, no albumin is present, and the cloudiness was due to precipitation of the earthy phosphates in urine deficient in acidity. A faint cloudiness remaining is due to mucus-proteins, entangled in the phosphatic coagulum. The cloudiness due to mucus is very faint. Any plainly perceptible cloudiness remaining after addition of acetic acid signifies albumin, especially if, on second boiling, it becomes more apparent.

D. When the acetic acid clears up the phosphatic coagulum at the top, a ring-shaped coagulum of these same phosphates may remain below, as yet not affected by the acid. This should not be mistaken for albumin.

E. As regards adding 50 per cent. acetic acid, drop by drop, unless the urine foams when the first drop of acid is added it

is not necessary nor desirable to add more than a drop or two of the acid. If the urine foams, add the acid until the foaming at the top ceases, *i. e.*, until the alkalinity is overcome. This applies to 10 c.c. of urine in a slender test-tube.

The principle of the test is that heat coagulates albumin in the presence of neutral salts in the urine, provided the urine is sufficiently acid, and not too acid. Boiling fails to coagulate albumin in alkaline urine since alkali-albuminate is not precipitated until the solution is neutralized, hence the object of careful acidulation. Too much acid must not be added for fear of forming acid-albuminate, which in turn is not coagulated by heating.

The value of the test is largely a negative one, *i. e.*, in proving absence of serum albumin and globulin; its delicacy, according to the author, is 1:100,000 determined by experiments with the aid of Dr. Alfred Lewy, of Chicago. It is readily applied, convenient, and superior to the boiling test with nitric acid. (See below.)

☞ **The objection to the test** lies in the fact that the protein of mucus is coagulated by boiling and acetic acid. It is possible, also, that certain balsams taken by the patient may in the urine yield a resinous precipitate.

The urine of nearly all women is faintly positive to this test. Urine containing bacteria is faintly positive to the test.

In a strong reflected light a faint haze due to the protein of mucus may be seen in many cases.

☞ Hence the test, when slightly positive, is not well suited to life insurance purposes unless supplemented by certain other measures. Moreover, it fails to show albumoses (peptones) in the urine since they are soluble in boiling solutions.

The author advises, therefore, that it be used **first**, *i. e.*, as a preliminary test. If negative, it is sufficient to exclude presence of serum-albumin and globulin in pathological quantity, but not of albumoses; if positive, it needs further support, except when the coagulum is so heavy as to be unmistakable. If negative, further evidence is needed regarding the possible presence of albumoses.

When Ultzmann's test is positive, shaking with ether should dissolve the precipitate if due to resins, but the precipitate should be abundantly obtained by use of considerable urine, 30 c.c. or more in order to be certain of results.

In a case of multilocular cystic kidney the writer found a large amount of albumin coagulated by boiling, but soluble in acetic acid. The term aceto-soluble albumin has been given to such protein, but its presence in urine has been denied by some authorities and ignored by others. In the author's case of cystic kidney, however, the protein described as soluble in acetic acid after boiling coagulated to a solid mass in the Esbach tube when the picric acid solution was added to it, thus proving it to be a protein and not merely "phosphates deposited by heat from feebly acid urine," as claimed by others.

II. THE HEAT TEST WITH SALT AND ACETIC ACID. (PURDY'S TEST.)

☞ The proteins of mucus (nucleo-albumin, mucoid substance) being coagulated by heat and acetic acid, it is necessary to provide a test for albumin which shall guard against error from this source. Mucus proteins do not yield a coagulum, however, unless the urine is poor in neutral salts, *i. e.*, sodium chloride; hence, by addition of the latter to the urine before the test is made the error may be avoided. To 10 c.c. of filtered clear urine add 2 c.c. of a saturated solution of pure sodium chloride, mix thoroughly, pour into a clean, slender tube of about 10 c.c. capacity, filling it three-fourths full, heat the upper portion, add the 50 per cent. acetic acid, drop by drop, as before (using from 2 to 6 drops), and boil again. Results as in the test described above.

The principle of the test is that only albumin and globulin are coagulated by boiling in acid urine rich in neutral salts. The proteins of mucus are not thus coagulated.

The value of the test is: (1) as confirmatory test when positive, and (2) when the urine of women is examined, as, *e. g.*, in a gynecological clinic it saves much time by excluding the proteins of mucus.

The objections to the test are: (1) it is apparently not as delicate as Ultzmann's test described above, and in suspicious cases, *in the* urine of men especially, cannot be relied upon altogether to negative a positive finding with Ultzmann's test. The microscope is indispensable in such cases for the detection of casts, since the author has found casts in urine positive with Ultzmann's test and negative with the salt test; (2) it fails to detect albumoses and may react with resins, (3) it requires time and care in measuring and mixing.

III. THE HEAT TEST WITH NITRIC ACID. (COTUGNO'S TEST.)

There are many who still use this test, now 140 years old, which may be performed as in the case of Ultzmann's. Boil the upper portion of the clear filtered urine and add pure colorless nitric acid of specific gravity 1.42, drop by drop, in amount from one-twentieth to one-tenth of the volume of urine used. If boiling causes a precipitate, the nitric acid added dissolves the phosphates, but does not affect the albumin, but the urine should not be further boiled after the addition of nitric acid. If boiling does not cause a precipitate, but one appears after addition of a few drops of acid, add a few drops more and any precipitate remaining is due to albumin. If a precipitate formed by boiling disappears when shaken after addition of a few drops of acid, but reappears after further addition of a few more drops of acid, albumin is present. If the test is negative, the tube is allowed to stand for a time and after cooling a trace of albumin may form as a precipitate at the bottom of the tube, but if such a precipitate redissolves on warming, it is due to albumoses and not to albumin.

Authorities are not agreed upon the best methods to employ, among which are the following:

Method I.—Fill a test-tube half full of clear urine, hold it with a test-tube clamp, boil, and add one-tenth its volume of nitric acid.

Results: if any cloudiness, coagulum or precipitate is seen after boiling and addition of nitric acid, albumin is present.

Method II.—Boil about an inch of the clear urine in a test-

tube and add 2 or 3 drops of 10 per cent. nitric acid solution. If after a few moments there is no cloudiness, coagulum, or precipitate, boil again, add ten drops more of the dilute nitric acid and set aside. A trace of albumin may become visible after an hour or so, not seen at first.

Method III.—Clarify the urine by addition of a few drops of potassium hydroxide solution, boiling, and filtration. Fill a test-tube half full of it and add 15 to 18 drops of strong nitric acid. If the urine contain albumin it will become somewhat cloudy. Boil and let stand half an hour. There will be seen a sediment of whitish flakes or granules. Boil again, when, if albumin be present, the flakes will not be dissolved by the second boiling.

Method IV.—Twenty-five per cent. nitric acid is used as in Ultzmann's test, instead of acetic acid, but in greater quantity, namely, 1 to 2 drops per 1 c.c. of urine. The urine should be boiled before and after addition of each drop of acid, and allowed to cool. A precipitate forming after the urine cools is either due to albumoses or to a trace of albumin. The author is inclined to favor this method.

Principle of the Test.—Albumin coagulated by boiling is insoluble in nitric acid when the latter is added in the proper proportions. Other proteins do not respond to the test except "nucleo-albumin," so-called, in large amount, while phosphates are dissolved. The precipitate is **flocculent** and is thus distinguished from the urates and resins.

Advantages of the Test.—It is said that "nucleo-albumin" in small amounts, as, *e. g.*, in the urine of women, does not respond to this test.

Disadvantages of the Test.—These are both physical and chemical. When the boiling urine is alkaline, nitric acid causes, when added to it, an inconvenient effervescence, an unpleasant odor, and the liquid, if it boil over, is corrosive and stains the hands and clothing. When the urine is not alkaline, though the above features may be absent, even three drops of the acid causes a change of color in the urine which may be mistaken for cloudiness by the inexperienced, since the color of the urine becomes

darker. The handling of the acid in the application of the test is likely to result in stains upon the clothing or burns on the hands.

Chemically, the use of nitric acid not in proper proportions may lead to error, since (1) traces of albumin are soluble in hot solutions containing a small amount of the acid, and (2) if a small trace of the acid is present in the tube through uncleanliness, before the test is made, it may convert the albumin into acid-albuminate not coagulable by heat, (3) in alkaline or neutral urine if too little acid be used, part of the alkali may still form alkali-albuminate with the albumin which is also not coagulable by heat, (4) if the nitric acid is added too liberally it will redissolve the albumin coagulated by heat, (5) "nucleo-albumin" in large amount gives a haze with this test, (6) coloring matters, when in excess, cause so great darkening so as to render it difficult to detect a trace of albumin, (7) nitric acid, unless freshly made, is likely to contain nitrous acid, which effervesces with all urine and may thus mask the reaction, (8) urates and resins may respond to the test; their precipitates are not, however, flocculent.

Clinical Note.—L. de Jager claims the following:

The boiling test of the urine does not always yield reliable results even when acetic or nitric acid is added afterward. The failure is due to the presence of calcium phosphate. Urine containing the latter may yield a positive result, or it may remain clear on being heated, although albumin may be contained in it. To prevent error, 1 c.c. of a solution of potassium oxalate (20 per cent.) should be added to the urine before this is boiled. After filtration the boiling is performed; if the urine is not rendered turbid, a few drops of acetic acid are added. If no precipitate occurs when the urine has been treated in this manner, then there is no albumin contained in it.

IV. HELLER'S TEST: COLD NITRIC ACID BY CONTACT.

This test is extremely popular owing to the readiness with which it may be performed. Into a small slender tube is poured about half an inch of cold, colorless nitric acid of specific gravity

1.42; the tube is held nearly horizontal in one hand (Fig. 20) while clear filtered urine is allowed to trickle down the side of the tube from a pipette held in the other hand until equal in amount to the acid. The tube is then **very slowly raised** to the perpendicular and allowed to stand undisturbed for several minutes. At the end of not less than three minutes the tube is held against a dark background and if a white ring is seen **exactly** at the juncture of the acid and the urine, the test is positive for serum-albumin, globulin, albumoses and resins. The more slowly and carefully the urine is poured in, the more compact and clear-cut will be the ring, hence the various forms of apparatus devised. The urine is preferably delivered from a medicine dropper with rubber nipple. (See, however, the conical glass method described below.)

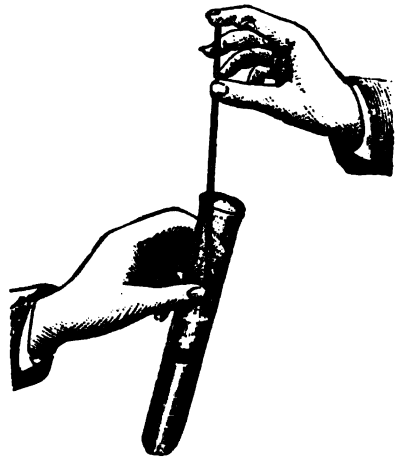


FIG. 20.—Heller's Nitric Acid Test.

The principle of the test is the precipitation of acid-albuminate, insoluble in excess of acid at the point of contact, while the protein of mucus being soluble in strong acid does not appear.

The advantages of the test are the readiness of application of it, the exclusion of traces of mucus proteins, and the positive reaction with albumoses.

The objections to the test are very numerous, so many, in fact,

as to confuse the inexperienced. The principal opportunities for error are to be found in the following:

1. Authorities differ as to the proper method of applying the test.

2. The test is not so delicate as Ultzmann's, the delicacy determined by Dr. Lewy and the author being 1:20,000 when urine is floated upon acid.

3. Minute quantities of albumin do not form a white ring, but merely a *ring-shaped haze* which will not form at all unless great care be used in applying the test, hence the delicacy in the hands of the hurried practitioner is likely to be much less than 1:20,000. The author has repeatedly been informed of the absence of albumin in specimens tested by inexperienced persons using this method, when presence of it could readily be demonstrated by careful methods of procedure.

4. A generally diffused haziness above the line of contact is not due to albumin, when care has been taken in the floating, but to other constituents. (See below.)

5. The white ring at the point of contact may be due to albumoses. Warming the tube with great care to avoid boiling the entire liquid at the zone where the ring is formed will dissolve it, if due to albumoses, and it will reappear on cooling, when the liquid becomes intensely yellow in color.

6. A cloudiness *not at point of contact,* but *higher up, more diffused* and spreading downward, is *not* albumin, but due to precipitation of *urates* or some other protein of unknown character, especially in urines of high specific gravity. In case of doubt perform the test again as follows: dilute the urine with two or three times its volume of water, warm the nitric acid before adding the urine, and now no cloud will be seen if the previous cloud was due to urates. Or use the conical glass method of Simon. (See below.)

7. A light cloudiness *near the surface* of the urine is *not* albumin, but due to nucleo-albumin, so-called, or other protein of mucus.

8. In all urines a *transparent zone of color,* more or less

intense, appears. This is *not* albumin, but is due to the oxidation of the normal chromogens of the urine by the acid. The color is violet, reddish, or brownish, and its transparency can be observed by holding the tube up to the light. When albumin is also present, the white ring is seen above the color zone.

9. A zone of slowly forming *crystals* may be observed in urines of high specific gravity containing 3 per cent. or more of urea. The crystals are *nitrate of urea* and will not form if the test be tried again with dilute urine and warm acid as in 6.

10. *A yellowish-white zone*, less plainly defined than the albumin ring, may be due to precipitation of certain resinous bodies, such as those contained in turpentine, balsam of *copaiba*, tolu, storax, cubebs, salicylic acid, etc., taken by the patient. Shake the urine-acid mixture with ether, which will dissolve the resins, but not affect coagulated albumin. Use also the wine glass method of Simon. (See below.)

11. If *blood* is present, the albumin ring will be colored *brown-red;* if bile a *greenish* or blue color-zone will be seen.

12. If the nitric acid contains *nitrous acid* as an impurity, bubbles will rise, even in acid urine, and may obscure the rings. These bubbles are probably due in acid urine to decomposition of urea by nitrous acid. In alkaline urine the bubbles are due to carbon dioxide liberated from carbonates present by action of the acid, and will, hence, always be seen, even if the nitric acid be free from nitrous acid, and no decomposition of urea take place.

13. According to some authorities, as C. E. Simon, the mucus proteins when present in amount more than traces can simulate the true albumin reaction. Hence, in doubtful cases dilute the urine with water and test again.

The chief practical objection to Heller's test is that it involves the use of a corrosive acid which burns and stains the hands and clothing. Novices with delicate skins sometimes suffer severely from nitric acid burns.

To overcome the objections applying to technique, various methods have been devised.

☞ 1. **The Use of Conical Glasses.**—C. E. Simon recommends the use of the conical glasses as follows: about 20 c.c. (5 fluidrachms) of the clear urine are poured into one of the glasses, and from 6 to 10 c.c. (1½ to 2½ fluidrachms) of nitric acid added by means of a pipette which is carried to the bottom of the vessel, when the acid is slowly allowed to escape by diminishing the pressure of the finger on the tube. (See Ogden's method below.)

Results.—If *albumin* is present a whitish ring is seen from below upward at the zone of contact. If albumin is small in amount, the upper border, in time, though at first as well defined as the lower, becomes less so, the cloudiness extending upward in the form of small irregular columns.

2. **The Pipette Method.**—A long, slender pipette is first dipped into clear filtered urine and about an inch of the latter allowed to rise in it, when the finger is placed over the upper orifice and the urine in it conveyed to a test-tube containing nitric acid in quantity. The finger is then removed and the nitric acid allowed to rise in the pipette below the urine in equal amount to the latter. The finger is then placed over the upper orifice and the pipette removed from the test-tube and inspected. The usual white ring is seen at the junction of the urine and acid. This method is recommended by Boston.

3. **H. Le Roy Thompson's Method.**—This is the simplest and readiest of all clinical methods known to the author: a glass funnel delivery tube is bent at an angle, the lower orifice cut off obliquely, and a rest blown in the tube at the angle where it is bent. The whole is set on the rim of a test tube containing the acid, a filter paper is placed in the funnel and the urine, as fast as it filters, trickles down the side of the tube and floats on the surface of the acid. Care must be taken to use a test-tube suited in size to the angle at which the funnel tube is bent, *i. e.*, a small tube cannot be used at all.

☞ 4. Nelson Baker & Co. supply an instrument known as the horismascope, a U-shaped tube provided with a black background. The urine is poured into the larger limb and the acid into the smaller underlying the urine. (Fig. 21.)

☞ **Laboratory Note.**—Ogden insists that the conical glass method is the only proper way of applying the cold nitric acid test, since by use of it the urine and acid *mingle* slightly, hence a trace of albumin is more rapidly detected; the ring of acid urates forms higher up and the precipitate of balsams below the juncture of urine and acid. But he differs from Simon in that he allows the acid to trickle down the side of the glass and condemns the pipette carried to the bottom.



FIG. 21.—Heller's Test with the Horismoscope.

V. THE FERROCYANIC TEST.

F. Engels deems this test secondary in value to that of heat and acetic acid. It is, however, of value in various quantitative analyses to determine whether all traces of proteins have been removed from the urine. Emerson thinks that any one expert in the use of it may profit by it, even in qualitative analysis. Much depends upon the proportion used, and the author agrees with Engels in regard to its comparative value.

Technique.—The clear, filtered urine is acidulated with 2 per cent. of its volume of 30 per cent. acetic acid. If a precipitate of mucoid, etc., occurs, the urine should be filtered. To the filtered or clear urine add, drop by drop, a 5 per cent. solution of clear potassium ferrocyanide from an all-glass pipette until 3 per cent. of the original volume of urine has been added. When the proper proportion of ferrocyanide has been added a flocculent precipitate shows albumin or albumoses. The test should be performed very slowly, waiting a few seconds after each drop has been added for fear of excess. The tube should be compared from time to time during the test with another tube containing clear, filtered urine. The test may be performed by the contact method as follows: add 5 drops of the ferrocyanide solution to 5 c.c. of 30 per cent. acetic acid and float on 5 c.c. of clear, filtered urine. The proteins of mucus interfere with this contact test, which is, therefore, not advisable.

Principle of the Test.—Proteins in urine acidulated by acetic acid are precipitated (not coagulated) by potassium ferrocyanide, the precipitate being soluble in excess.

Advantages of the Test.—One advantage of the test is that it is portable and does not require the use of heat. Moreover, the chemicals used are non-corrosive. If the urine be filtered after addition of acetic acid, mucoid, etc., is removed and does not complicate the albumin test. Albumoses are detected and in this respect the test is superior in range to the heat test. It does not precipitate alkaloids, phosphates, or peptones.

Disadvantages of the Test.—It is probably not so delicate as the heat and acetic acid test. The ferrocyanide solution soon becomes cloudy, unless care is taken to keep it in an amber bottle and away from possible traces of iron, uranium, copper, etc. Nipple pipettes can not be used with it on account of the oxide of zinc in the rubber. It is frequently necessary to dilute the urines in order to obtain a precipitate of albumin or albumoses, hence, care must be observed in testing urine of high specific gravity. The test is slow. The above disadvantages are covered in the following:

Precautions to be Observed.—1. The ferrocyanide solution should be freshly made in pure distilled water, filtered clear, kept in a clean bottle, with clean stopper, away from the light.

2. It is well to see that the acetic acid and the ferrocyanide solution, when mixed together *without urine*, remain clear.

3. The writer has noticed that, if the acetic acid be added from a pipette with a *rubber nipple*, a cloudiness or precipitate due to zinc salts in the rubber sometimes takes place. If pipettes are used they should be all glass.

4. Heating will cause a precipitate in the mixture without addition of urine.

5. When the amount of albumin is very small, cloudiness is not seen immediately after adding the urine, *but only after a few minutes have elapsed.*

6. The ferrocyanide test precipitates albumin, globulin, acid and alkali-albumins, albumoses; nucleo-albumin from bile is said to be precipitated by it, and also mucoid substance (nucleo-protein, mucus-protein).

7. Urines of high specific gravity should be diluted with water lest albumin and albumoses be not precipitated.

8. Removing the coagulated substance with a pipette, and boiling it, will tell whether it is albumin or albumoses, since the latter clears when boiled and reprecipitates on cooling.

9. Partial clearing on boiling indicates a mixture of albumin with albumoses, but care during the process is necessary, since boiling decomposes the ferrocyanide solution and makes it cloudy. The best way to manage it is to let the precipitate settle thoroughly, decant supernatant liquid, add water, let settle again, and remove the pipette. This can be done rapidly by the use of the centrifugal machine.

In the author's mind the principal objection to the ferrocyanide test is its wide range, so many different substances being precipitated by it that when it comes to faint hazes we are not always sure just what they represent.

Laboratory Notes.—1. Purdy, in his quantitative method, uses a 10 per cent. solution of potassium ferrocyanide and 50 per cent. acetic acid. (See below.)

2. Another method of applying the test is as follows: into the bottom of a *clean* test-tube, pour 15 to 30 drops of acetic acid (30 per cent.), then add two or three times that amount of a 5 per cent. ferrocyanide solution and shake. The mixture should remain clear. Now add clear filtered urine, and, if albumin be present, it will be precipitated throughout the whole volume of the urine in the form of a more or less milk-like flocculent cloud, according to the quantity of albumin present.

3. H. E. Monroe, of Mattoon, Ill., has a good method as follows: take two test-tubes and place a small quantity of urine in each. Dilute each volume of urine with one-third its volume of water. Add a few drops of acetic acid to one tube only and note by comparison with the other tube whether any precipitate of nucleo-albumin, so-called, etc., takes place. Now mix the contents of the two tubes, pouring from one to another several times; finally divide the total volume of urine again so that half is in each tube and add to one tube, only, one or two drops of a 10 per cent. ferrocyanide solution, comparing results with the liquid in the other tube.

Clinical Note.—Langstein employs the ferrocyanide test in the diagnosis of orthostatic albuminuria in children as follows: the filtered urine is placed in two test-tubes to the same height and a few drops of moderately diluted acetic acid are added to each tube. After shaking the contents of both for a few minutes, some distilled water is added to each tube and a few drops of weak solution of potassium ferrocyanide to one tube only. The less the difference in the turbidity of the two tubes the greater the probability of the existence of a genuine orthotic albuminuria. (For the positive diagnosis the blood pressure and the condition of the eye must be known.)

MISCELLANEOUS TESTS.

A large number of tests for albumin have been proposed. Some of them may be of service for special purposes, hence are here described.

Spiegler's Test.—This is the most delicate known test for proteins. The formula of Jolles is mercuric chloride 10 grammes,

succinic acid 20 grammes, sodium chloride 10 grammes, distilled water 500 c.c. The urine after filtration is acidulated with acetic acid and filtered again to remove mucoid, etc., then superimposed on the chemical solution. A sharp white ring shows albumin, or albumoses (not deutero-albumose).

Tanret's Test.—This is a favorite with the French and is extremely delicate. The solution consists of mercuric chloride 1.35 gramme, dissolved in as little water as possible, together with 3.32 grammes of potassium iodide. After the two are dissolved, 50 c.c. of water are added and then 20 c.c. of glacial acetic acid. Some writers advise dissolving the salts separately and then mixing. The test may be made in two ways, either by adding the solution, drop by drop, to the urine until a cloud is seen, or by superimposing the urine in the solution. A large number of substances are precipitated; mucoid, albumoses, peptones, vegetable alkaloids, etc., as well as albumin. Gently heating the urine before or after superimposing will prevent or dissolve the precipitates due to albumoses or alkaloids.

Robert's Nitric-Magnesium Test.—Ten c.c. of pure nitric acid are added to 50 c.c. of a saturated solution of magnesium sulphate and the test applied by contact. A sharply defined, dense, rapidly appearing lower ring shows "clinical albuminuria," hence the test is a convenient one for eliminating resins. Mucoid, etc., in normal urines is not precipitated, but in large amount reacts. This test causes a cloudiness in urines first passed after an ejaculation of semen. Heating the clouded urine after addition of the reagent distinguishes serum albumin from the albumoses.

The crystals may be used as a test by dropping them in small quantity into the urine with shaking, hence an advantage is obtained as regards portability. The test fails in alkaline urine, hence such urine must be rendered acid by acetic acid.

Johnson's Picric Acid Test.—A cold, saturated solution of picric acid (0.5 gramme in 30 c.c. water) is superimposed upon clear filtered urine previously acidulated with citric or acetic acid. A yellowish white zone of precipitated albumin is seen at the line of contact. If the urine be previously gently heated, alkaloids,

mucoid, and urates are not precipitated: otherwise they complicate.

Roch and MacWilliams' Sulphosalicylic Test.—A saturated solution of salicylsulphonic (sulphosalicylic) acid is added to clear acid filtered urine, in proportion 1 or 2 drops of the reagent to 20 of the urine. On shaking the tube, a uniform cloudiness immediately appearing shows albumin, globulin, albumoses, and peptones, but not resins, alkaloids, or mucoid.

Other tests are the *trichloracetic* saturated solution by contact, *potassium sulphocyanide* (a few drops of 100 c.c. of a 10 per cent. solution acidulated with 20 c.c. of acetic acid added to urine; or succinic acid and potassium sulphocyanide mixed in solid form and a little added to the urine); *sodium tungstate*—equal parts of 20 per cent. sodium tungstate solution and saturated citric acid applied by contact; *mercuric chloride* with acetic or citric acid (addition of a few drops of 10 per cent. corrosive sublimate solution to urine followed by a few drops of acetic acid or a mixture in capsules of mercuric and sodium chlorides with citric acid added to urine); *phenol-acetic* (Millard's modification of Méhu's) a mixture of glacial carbolic acid 20 gm., glacial acetic acid 60 gm., and liquor potassæ 220 grammes applied by contact; *resorcin* (Carrez) floating the urine upon a solution of 1 part resorcin in 2 of water; *acidulated brine,* urine floated on a mixture consisting of 30 c.c. of pure hydrochloric acid in 500 c.c. of a saturated solution of sodium chloride; *metaphosphoric acid* in solid form, adding a fragment the size of a pea to a test-tube full of clear, filtered urine; *chromic acid,* 1 part of a 5 per cent. solution with 3 parts of urine, the upper portion boiled, if necessary; *betanaphthol-sulphonic,* 5 c.c. of urine heated with 20 or 30 drops of a filtered solution of 10 grammes of the reagent in 200 c.c. of water, boiling to exclude albumoses or urates.

Inexperienced persons should avoid dabbling with these tests. If any one test be tried, it should be given a long and thorough consideration in comparison with some of the five ordinary clinical tests above described.

CHAPTER XVIII.

THE QUANTITATIVE DETERMINATION OF ALBUMIN.

Esbach's method for quantitative determination of albumin.
Advantages and disadvantages of Esbach's method.
Tsuchiya's phosphotungstic method; technique, advantages, disadvantages.
Purdy's centrifugal method; technique, advantages, disadvantages.
Relation of the centrifugal method to the Esbach method.
Cases from practice illustrating fallacies of the methods.
Aufrecht's centrifugal method.
"Ring" determination of albumin.
Titration methods: Goodman and Stern's; Harrower's tube.
Vassilieff's sulphosalicylic method.
The gravimetric determination of albumin; Salkowski's and others; the densimetric method; the method by refractive index.
Removal of albumin from urine.
Relation of albuminuria to acidity.

There is no ready clinical method for the determination of albumin sufficiently accurate to appeal to the chemical mind.

☞ The favorite *clinical method* is that of *Esbach*, which involves use of the *Esbach tube* (Fig 22), a specially constructed tube which has an upper mark R, a second mark below it, U, and the figures 7, 6, 5, 4, 3, 2, 1, one above the other, indicating graduations of the tube, in parallel lines, beginning just below U, and going down to nearly the bottom of the tube. Between the mark 1 and the curved bottom of the tube is a short mark, which is $\frac{1}{2}$ of 1.

Another form of the tube is that with a tapering extremity and foot, allowing guesses at smaller quantities of albumin than the one with original curved extremity.

Technique of Esbach's Method.—Unless the urine is strongly acid add acetic acid, drop by drop, to it until it turns litmus paper a brick red. The tube is then filled to the mark U with urine which must not exceed 1010 in specific gravity, hence in most cases requires to be diluted and the dilution included in the cal-

culation. Then fill to the mark R with Esbach's reagent, a liquid made by dissolving 155 grains (10 grammes) of picric acid and 310 grains (20 grammes) of citric acid in 30 fluidounces (900 c.c.) of distilled water and, after solution is accomplished, adding enough distilled water to make the total one liter (1.05



FIG. 22.—Esbach's Albuminimeter.

quart, or a little over 33 fluidounces). The solution should be made in cold water and the chemicals powdered in a mortar before solution is attempted.

After the yellow reagent fluid has been added, close the mouth of the tube with the cork and invert a dozen times **without shaking.** Let it settle for 24 hours. The precipitated proteins, if

present, settle down to the bottom of the tube, and the height of the deposited mass may be measured by the lines, 1, 2, 3, 4, etc., on the outside of the tube. The originator, Dr. Esbach, claims that these lines indicate percentages of albumin by *weight, viz., 1-10, 2-10, 3-10, etc., of 1 per cent. by weight, i. e.,* 1, 2, 3 grammes per liter, etc. These figures must be carefully distinguished from the old-fashioned method of reckoning roughly *by bulk,* namely, 10 per cent., 20 per cent., etc., after boiling with heat and nitric acid. One per cent. of albumin *by weight* is a very large quantity, but 1 per cent. *by bulk* is an exceedingly small quantity, not much more than a plain trace. The Esbach tube is graduated so as to express percentages by weight, not bulk, and this must not be forgotten. For clinical purposes discard close reckoning by the Esbach tube save in the following way:

1. The precipitated proteins settle down below mark 1: *albumin is small in quantity;* very small, if below the ½ mark.

2. The precipitated proteins settle down below 3, but above 1: *albumin is moderate in quantity.*

3. The precipitated proteins settle down to any figure or letter above 3: *albumin is abundant;* very large in quantity if 5 to 7; enormous if much above 7. In such cases dilute the urine with an equal volume of water and multiply. In general it is better to dilute the urine so that its specific gravity does not exceed 1008 and multiply the figure obtained by the total number of volumes of urine and water used, *i. e.,* by 2, if equal parts of urine and water, by 3, if two parts water, etc.

Principle of the Method.—The picric acid solution precipitates albumin and the relation of the bulk of the precipitates to the results of gravimetric determination of albumin in any given sample is indicated by the figures on the tube. That is to say, given a urine from one liter of which one gramme of albumin may be obtained by boiling, filtering, washing, drying and weighing, and this same urine in the Esbach tube to the mark U with reagent to the mark R will give a precipitate which in 24 hours will settle to the mark 1.

Advantages of the Method.—The principal advantage of the method is the ease with which the determination may be made, so that students, nurses, and even patients can readily be taught the use of it. This no doubt accounts for its popularity. Then again, the tube and the reagents are inexpensive.

☞ **Disadvantages.**—These are many, and from a scientific point of view the method is often childish, but occasionally surprisingly accurate. If a number of Esbach tubes be prepared according to Esbach's method by a number of different individuals, the results may show considerable discrepancy. Then again, if a round tube be compared in results with a tapering one, a discrepancy may be noticed. Again, in a warm room the precipitate settles quickly, in a cold room more slowly. The most trustworthy results are obtained between the marks 1 and 4, hence the desirability of diluting a urine loaded with albumin. The room in which the tube stands must be kept at nearly a constant temperature; about 60° Fahrenheit is recommended. The same urine may indicate 1 gramme per liter of albumin in one tube, or as high as 3 grammes in another, or at another time, hence the desirability of reckoning the amount very small, small, medium, large, very large, and enormous, according to the author's plan. The author has seen a sample in which the tube with the tapering end and the foot showed only ½ gramme per liter, while the round one without a foot indicated 1.25 grammes per liter, the real amount obtained gravimetrically being 1.75 grammes per liter.

In some cases the "precipitate" floats on the top of the liquid, hence it is desirable to watch it for a time and if this tendency to float be observed stir gently with a glass rod in order to overcome it. It is said that dilution of the urine also prevents this trouble.

The mixture of urine and solution must not be shaken and should be reversed slowly for only a dozen times, lest some of the precipitate be redissolved.

In strongly alkaline urines acidulation with stirring must be made as directed above *outside the tube*, on account of foaming.

Traces of albumin will not settle at all, but render the entire liquid cloudy.

The same is true of mucoid, and when the latter is present in large amount the bulky mass fails to settle.

If the patient is taking balsams (copaiba, sandalwood) an error is caused, since picric acid precipitates the resinous acids; also alkaloids, as quinine, morphine, antipyrine, etc.

Since the greater number of cases in which we find albumin in the urine are those where a very small percentage is present, if we wish to record figures the Esbach tube is practically useless in the majority of cases. The term trace should, strictly speaking, be applied only to that amount which clinically we are unable to weigh or to measure.

Tsuchiya's Phosphotungstic Method.—The author in some cases prefers the method of Tsuchiya to that of Esbach; his solution as now made consists of 1 gramme (not 1.5) of phosphotungstic acid in crystals in 100 grammes of 96 per cent. alcohol and 5 grammes of hydrochloric acid of specific gravity 1.19. Dilute the filtered urine to be examined until it has a specific gravity of 1006, or not above 1008. Use urine and reagent with a special tube, filling, etc., as in the case of the Esbach method. Tsuchiya has devised a special tube in which the proportion of urine to reagent is 9 : 8, and which is more accurate than the Esbach tube.

Advantages.—The precipitate usually settles better than that of the Esbach method, and the results in different tubes at different times are not so variable. For example, in the case of the urine mentioned above which showed ½ of 1 gramme per liter in the tapering Esbach tube and 1.25 grammes per liter in the round one, with the Tsuchiya fluid it showed exactly the same in both, viz., 1.5 grammes per liter, even in the undiluted urine.

The bulk of the precipitate remains almost the same for many hours after it has once settled.

The precipitate does not tend to float as often as in the case of the Esbach process. Sugar does not interfere. The solution does not stain the hands and has an agreeable odor. It is more easily prepared than the Esbach reagent. Slight variations in

the amount of albumin may be shown more readily than by the Esbach method.

Disadvantages.—In spite of the claim of Mattice and others there are certainly some urines rich in albumin which require dilution before the precipitate will settle. There is sometimes, also, a tendency for a break to occur in the precipitate as it settles. The special Tsuchiya tube must be imported, as it is thus far not made in this country. O. Schiemann insists that Tsuchiya's method possesses no especial advantages over that of Esbach except when the amount of albumin is large, *i. e.*, above 6 per liter. For very small amounts of albumin he claims it is inferior.

☞ **Purdy's Centrifugal Method.**—This clinical method is popular among those who possess a centrifugal machine. A Purdy centrifuge is required with an arm such in length that the distance from the center of rotation to the tip of the tube is 6¾ inches. The centrifuge must revolve at a uniform speed of 1500 revolutions per minute. Special percentage tubes (Fig. 14) are used holding 15 c.c. provided with tapering extremities, and graduated in tenths of a c.c. Fill a tube with clear filtered urine up to the 10 c.c. mark, add 2 c.c. of 50 per cent. acetic acid and 3 c.c. of a 10 per cent. solution of potassium ferrocyanide and mix thoroughly. Set aside for ten minutes, then place in the aluminium guard with a companion tube similarly treated and sediment at 1500 revolutions per minute for three successive periods of five minutes each. Read off the bulk percentage indicated by the height of the precipitate in the tube.

Each 1/10 c.c. of precipitate may be designated as 1 per cent. by bulk of albumin and each per cent. by bulk of albumin equals 0.021 per cent. by weight. Thus, 1 per cent bulk = 0.021 by weight; 2 per cent. bulk = 0.042 by weight; 3 per cent. bulk = 0.063 per cent. by weight; etc., etc. Each per cent. by bulk represents one-tenth of a grain of albumin per fluidounce, *i. e.*, 1 per cent. 0.1 grain, 2 per cent. 0.2 grain, etc. Hence, 48 per cent. by bulk corresponds to 1 per cent. by weight, or 4.8 grains per fluidounce.

Laboratory Note.—One per cent. by bulk of albumin is, then,

a very small quantity, but 1 per cent. by weight is a very large quantity, the latter corresponding to nearly half the bulk of the urine.

If the precipitate does not settle, dilute the urine with water and try again.

Principle of the Method.—Dr. Purdy found that a sample of urine, which yielded by gravimetric analysis, for example, one grain of dried albumin per fluidounce (2 grammes, nearly, per liter), when precipitated in one of his tubes with acetic acid and potassium ferrocyanide in proper proportions, showed an albumin precipitate having a bulk of 10 tenths (1 c.c.) after being sedimented at a certain speed for a certain time.

Advantages of Purdy's Centrifugal Method.—The rapidity of the method, requiring as it does only 25 minutes in all, is the principal advantage; again, the method is advantageous for recording small quantities of albumin which can not be estimated closely by use of the Esbach tube.

Disadvantages.—These are both mechanical and chemical. The speed of an electric centrifuge varies according to the time of day, being less in the morning and greater in the evening. The precipitate shrinks measurably according to the speed and time, hence an error may be occasioned unless the precise speed be attained. An error may also result from the length of the radius, which must be six and three-quarter inches.

☞ Clinically, the principal disadvantages of the method are: (1) that quantities of albumin above 10 per cent. by bulk do not always settle well, and (2) mixtures of mucoid, nucleo-albumin, etc., with albumin, interfere with the settling.. Dilution with water in some cases is useless, since even diluted urines may fail to settle or when diluted fail to correspond properly in bulk. In some cases urines containing albumin in amount less than 10 per cent. by bulk, diluted with equal parts of water, will show 6 or 8 per cent. by bulk both before and after dilution. Again, some urines require so much dilution with water in order to settle at all that multiplication by the number of volumes used results in a percentage figure above 48, which is obviously incorrect.

Still further, in some cases, dilution with a few volumes of water, as, *e. g.*, 2 or 3, may result in perfect sedimentation when albumin is very large in amount in the urine, but the bulk percentage obtained by multiplying will be obviously too great, figures between 100 and 200 per cent. being obtained, while further dilution with more volumes of water may result in imperfect sedimentation, the precipitate in a 1:10 or 1:20 dilution not settling sufficiently well to read off. These criticisms, however, apply to a speed of 1,000 revolutions per minute only.

Urates may also be precipitated along with albumin in the highly acid urine and add to the bulk of the precipitate. If this is suspected, the supernatant fluid may be withdrawn from the sedimented mass by use of a pipette, the tube filled with hot water up to the mark 15 and after shaking well placed in the centrifuge and sedimented over again.

Albumoses and mucoid also interfere with the correct bulk percentage reading.

Relation of the Centrifugal Method to the Esbach.—In three cases of albuminuria the author found that the percentage by the Purdy method at 1,000 revolutions of 10, 11, and 14, respectively, all showed 1 gramme per liter, 0.1 per cent. by weight, on the Esbach tube. According to Purdy's table they should have shown from 0.2 to 0.3 on the Esbach tube, at a speed of 1,500 revolutions when the centrifugal method was used.

AUFRECHT'S ALBUMINOMETER.

☞ Aufrecht, recognizing the inaccuracies and discrepancies of the Esbach method, has devised an albuminometer for centrifugal use consisting of a cylindrical reagent tube (Fig. 23) closed and narrow at the bottom and graduated. The uppermost letter R, next U, and lower down the figures 1.7 per cent., 1.6 per cent., down to 0.01 per cent. The reagent consists of 1.5 gramme picric acid and 3 grammes citric in water to make 100 c.c. For use the tube is filled to the U with acid urine and then to R with the reagent. The tube is closed with the rubber stopper and the contents gently mixed, after which it is placed in the centrifugal

machine and centrifuged. According to the speed of the machine the time required is 2 minutes for 5,000 revolutions per minute, 2½ minutes for 3,000, and 3 minutes for 2,000 or 2,500. For a speed of 1,000 revolutions, not less than 6 minutes should be used. But the results may be higher than the Esbach tube gives.

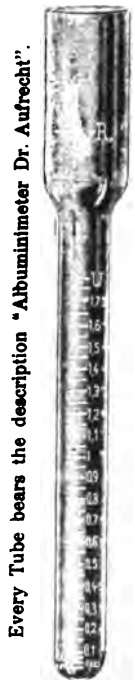


FIG. 23.—Aufrecht's Albuminimeter.

After centrifuging the percentage is read off from the height of the precipitate. The method possesses the great advantage that no dilution of the urine is required except in the extremely rare cases when the percentage is above 1.7 by weight.

The author, using Aufrecht's method, finds that it is necessary, in order to obtain constant results, to measure the speed of the centrifugal machine carefully so as to know how long to centrifuge, since a minute or two longer than three minutes, for

example, will influence the reading considerably. Thus I obtained a reading of 0.425 per cent. in 3 minutes, but 0.375 per cent. in five minutes at the same speed, *i. e.*, 1,000 revolutions.

For use in the Purdy centrifuge it may be necessary to remove the rubber stopper provided and to substitute a short cork.

Albumoses may be reckoned by boiling 4 c.c. of urine, filtering and pouring the cooled filtrate into the Aufrecht tube. Absolute alcohol is then added to the letter U and the mixture centrifuged as before for two minutes. (The speed required for the two minutes is not stated, presumably 5,000.)

Comparison of Results.—The author, using the same sample of albuminous urine with Purdy's, Esbach's, Tsuchiya's and Aufrecht's method, obtained the following, at a speed of 1,000 revolutions:

Purdy's centrifugal method: at end of first five minutes no settling. Allowed to stand ten minutes then centrifuged again for five minutes and obtained a reading 20 per cent. by bulk. Esbach's method in tube with rounded end, 0.25 per cent. by weight at end of 24 hours. Tsuchiya's method, 0.35 per cent. by weight in 24 hours.

Aufrecht's method, 0.425 per cent. by weight at the end of three minutes, 0.375 at the end of five minutes, speed, 1,000 revolutions per minute.

☞ **Advantages and Disadvantages of Aufrecht's Method.**—Using a speed of 1,000 revolutions per minute as determined by the odometer to be had of Orr & Lockett, Chicago, the author has found that six minutes, at least, appears to be the proper amount of time for sedimentation when the Aufrecht method is to be used. Occasionally, at that speed, dilution of the urine has been necessary, and occasionally, mucoid or other mucus-protein has prevented complete sedimentation at that speed when the amount of protein was small.

A great objection to the Aufrecht tube is the need of a magnifying glass to read the finely engraved figures denoting small quantities of protein.

Again, at the speed of 1,000 per minute, very small quantities

are not as perfectly sedimented as by the Purdy ferrocyanide method at the same speed.

A great objection also to the Aufrecht method is the staining of the hands by the picric acid solution. Use strong solution of lithium carbonate to remove stain.

A speed of 1,000 revolutions per minute should be used for all methods tried, since this speed can be readily obtained by almost any centrifuge, hand, water, or electric, whereas high speeds are only occasionally available.

The author has tried the Tsuchiya phosphotungstic solution in the Aufrecht tubes and finds that it possesses an advantage over the picric acid solution of Aufrecht in that the precipitate is more flocculent, hence is higher and the figure corresponding can be read off with less difficulty. In such cases as have been compared with the Aufrecht liquid the height of the albuminous precipitate with phosphotungstic acid has been 1.6 times more than that of the picric acid precipitate, when sedimented for six minutes at 1,000 revolutions per minute.

"Ring" Determinations.—Efforts have been made to determine the amount of albumin by observation of the ring formed in Heller's test by contact.

(1) The urine is diluted until it takes about three minutes for the ring to appear with careful testing. Multiplying the number of volumes used by 0.003 gives the per cent. of albumin present. Thus, if 1 part urine was diluted with 5 parts water, the amount of albumin present is 0.18 per cent. There should be no nitric acid on the sides of the tube and the urine should be floated on the acid very slowly with a pipette. The same amount of acid and urine should be used each time for comparative tests and the same amount of time and care employed in floating the urine on the acid.

(2) Ogden uses a Collamore wine glass half full of urine and underlays this with one-third its volume of nitric acid; if the amount of albumin is as large as ½ of 1 per cent. by weight the ring is very dense and flocculent; if 0.25 per cent., quite flocculent from the side and opaque from above; if 0.125 per-cent.,

the bottom of the glass can not be seen, but a faint ray of light is transmitted; a large trace, 0.10 per cent., is a clearly seen ring, not flocculent, quite dense, but not opaque.

Trace is the term Ogden applies in this method, to a zone seen from the side without a dark background; slight trace, the same, only a faint cloud seen from above; very slight trace best seen with dark background, and slightest possible trace seen only by adjustment of a dark background obliquely in front of or a little to one side of the glass.

Titration Methods.—There are several methods for determining albumin volumetrically: *Goodman and Stern* use Tsuchiya's first devised solution, for which Harrower, of Chicago, has devised a specially constructed tube. The principle of the method is the determination of the amount of albumin necessary to cause the first sign of cloudiness with the solution. Five c.c. of Tsuchiya's original solution are used and filtered urine added, drop by drop, until a cloud appears on shaking. The tube, with directions for use, can be had from Dr. Harrower. The difficulty in this process is with the end-reaction, as it takes a quick eye to see just when the cloud appears.

Vassilieff's Method With Sulphosalicylic Acid.—This method is recommended by Boston for its ease of application. A solution (25 per cent.) of salicyl-sulphonic acid is made and poured into a burette. Ten c.c. of clear filtered urine, acidulated slightly with acetic acid, diluted to 50 c.c. with water and treated with 2 drops of a 1 per cent. aqueous solution of the yellow dye known as Echtgelb, are titrated with the acid until the mixture turns a brick-red color which is persistent. Each c.c. of the acid solution used corresponds to 0.01 gramme of albumin in 10 c.c. of urine. The method, in the author's hands, appears to give fairly good results when the quantity of albumin is large, but when small in one or two instances yielded figures which were obviously incorrect. The reagent is hard to obtain in this country.

THE GRAVIMETRIC DETERMINATION OF ALBUMIN.

There are a number of methods employed:

(1) *For large amounts of albumin* the method of Salkowski

is advised: 10 c.c. of urine accurately measured are mixed with from 10 to 20 volumes of 95 per cent. alcohol and brought to boiling on the water-bath. It is then cooled, allowed to settle, decanted, washed with hot water, partly decanted again, filtered, ashed on the filter, placed in a weighed platinum crucible and dried to constant weight at 115° C. (240° F.). The weight of the filter ash is then subtracted and the percentage of albumin obtained by multiplication by ten.

Clinical Note.—It is probable that the maximum amount of albumin present in urine is not above 4 per cent. by weight. Anything above 1 per cent. is a very large quantity.

For smaller amounts of albumin, so that not more than 0.3 gramme will be deposited on the filter, the following method may be used:

(2) Add to 500 c.c. of filtered urine one-tenth its volume of saturated solution of common salt and about 5 c.c. are boiled, cooled and filtered. Test the filtrate for albumin with acetic acid and potassium ferrocyanide and, if clear, proceed to boil the entire amount of urine. If turbid on application of the ferrocyanide test, add two or three drops of 50 per cent. acetic acid to the whole 550 c.c. of mixture made as above, stir well, and test again. Continue cautious addition of acetic acid to the whole until the filtrate remains clear when tested with the ferrocyanide test. Then heat the whole, first over the water-bath and then over the free flame, until the precipitate is flocculent and the supernatant fluid is clear. Filter through a dried and weighed filter, wash chlorine-free with hot water then with alcohol and ether, and dry to a constant weight at 115° C. (240° F.) in a weighing glass with a ground glass stopper or between clamped watch glasses in a drying oven on an asbestos sheet. Subtract the weight of the filter from that of the whole to obtain the weight of the albumin.

(3) Instead of the weighing process the precipitate obtained as above is collected on a small filter, washed as above, and the nitrogen estimated by the Kjeldahl method, multiplying the result obtained by 6.3 to obtain the albumin; or the total nitrogen

in the same volume of urine may be determined before and after coagulation and filtration, and the difference multiplied by 6.3 will show the amount of albumin.

(4) *The densimetric method* by the use of H. Zahn's coefficient is as follows: Ascertain the proper amount of acetic acid necessary to add to the urine in order for all protein to be coagulated by boiling, using the ferrocyanide test on the filtrate of a test-specimen. Then take the urine to be examined, acidulate it with the proper amount of acetic acid and place it in a flask firmly closed with a rubber cork. Immerse the flask for 10 or 15 minutes in the boiling water-bath. Filter. After filtration take the specific gravity of the filtrate and also of the urine itself, to which the same amount of acetic acid has been added without boiling, being careful that both fluids are at a temperature at which the urinometer is standardized. The difference in the two specific gravities multiplied by 400 gives the amount of albumin in grammes (?) per 100 c.c. of urine. This method is not difficult of execution, but the coefficient given in the books is obviously incorrect, since a difference of one degree in specific gravity would, according to the various authorities (?), indicate 400 grammes of albumin per 100 c.c. of urine.

(5) By use of the Zeiss immersion refractometer the index of refraction of the urine can be determined before and after coagulation of the albumin, and the amount of albumin thus calculated.

☞ **Removal of Albumin From Urine.**—In order to test the chlorides, etc., albumin must be removed. It is usually sufficient to boil the urine, adding acetic acid, drop by drop, until the precipitate is flocculent and the filtrate clear, water being added to bring the urine up to its original volume. In Hofmeister's process in sugar-free urine excess of 40 per cent. sodium acetate solution and a strong solution of ferric chloride are added until the whole mixture is of a red color, when after faintly acidulating it is boiled and filtered.

CHAPTER XIX.

VARIOUS PROTEINS: GLOBULINS, ALBUMOSES, MUCOID, FIBRIN, ETC.

Serum globulin defined.
The albumin quotient.
Tests for globulin:—the "streak" test; the test with alkali and "salting;" Daiber's method.
The quantitative determination of globulin:—Esbach method; alkali-and-salting method.
Clinical significance of globulin compared with albumin.
Clinical note on the globulin quotient.
Albumoses (propeptones); secondary albumoses and the Bence-Jones body.
Source of the albumoses.
Clinical significance of the albumoses.
Clinical significance of the Bence-Jones body.
Tests for albumoses: Heller's test; the biuret test; Alder's method; test for albumin and albumoses together.
Hofmeister's method and Bangs' method for testing the presence of albumoses.
Tests for the Bence-Jones body: simple clinical tests; the biuret test; Boston's sulphide test.
Quantitative determination of the Bence-Jones body by the Esbach method.
Mucoid and so-called nucleoalbumin:—the mucous cloud; Moerner's body; euglobulin.
Chemistry of the mucins: mucoid; Moerner's body; true nucleoproteins.
Physiology of mucoid and of Moerner's body.
Clinical significance of mucoid and of Moerner's body.
Relation of catarrhal conditions to mucoid in the urine.
Clinical significance of the nucleoproteins.
Clinical test for mucoid.
Clinical test for Moerner's body:—Heller's test; acetic acid test; magnesium sulphate test; citric acid test; Ott's test with tannic acid.
Fibrin:—occurrence; chemistry; clinical significance.
Detection of fibrin:—differentiation from mucopus and phosphates.
Nucleohiston and histon:—significance and isolation; tests.
J. E. Dale's systematic clinical scheme for identification of proteins.

Serum-globulin (para-globulin) is a general term for a group of proteins, namely, euglobulin, pseudo-globulin and fibrin-globulin, according to Hofmeister. The term *albumin quotient* is used to designate the value obtained in dividing the amount of serum-albumin by the total globulin. The latter is always present along with albumin in proportion varying from 8 to 60 per cent., and occasionally when albumin is absent. It is not present in normal urine, although Hofmeister claims that two members of the group, namely, euglobulin and fibro-globulin (fibrinogen) are probably always present in normal urine.

Tests for Globulin.—(1) The simplest clinical test is the "streak" test: to a beaker full of distilled water add a drop or two of albuminous urine. Each drop as it falls is followed by a milky streak or train if globulin is present, and finally an opalescence is seen throughout the whole urine. The cloudiness is soluble in acetic acid. Globulin is held in solution in the urine by the neutral salts present, but is insoluble in water or in highly dilute saline solution, hence is precipitated by an excess of water.

(2) Render the urine alkaline with ammonium hydroxide solution to precipitate phosphates, filter, and add an equal volume of a saturated solution of ammonium sulphate. Globulin and albumoses are precipitated. Wash the precipitate with half-saturated ammonium sulphate until the filtrate no longer reacts with ferrocyanide test. Dissolve the precipitate in a 1 per cent, solution of sodium hydroxide, acidify with acetic acid, and boil. A precipitate of globulin occurs, insoluble in saturated sodium chloride solution, since globulin, though soluble in weak salt solutions, is insoluble in strong ones.

(3) Daiber separates globulin from albumin as follows: the urine is poured into a vessel and mixed with an excess of absolute alcohol, which precipitates the albuminous substances. This mixture is left to settle for some hours, then filtered and washed with lukewarm, distilled water, then the deposit, together with the filter paper on which it is collected, is deposited in another vessel and distilled water at 30° C. (86° F.) is added, and, drop by drop, diluted acetic acid up to complete dissolution of the albumi-

noid substances. After filtration a solution of 1 part sodium carbonate in 4 parts distilled water is added, until the solution becomes perfectly neutral or slightly alkaline, and then a 50 per cent. solution of ammonium sulphate, which precipitates the globulin in the form of a flaky, white deposit. This latter may be filtered off and dissolved in a 1 per cent. solution of sodium chloride from which it may be again precipitated when the liquid is treated with ammonium sulphate. The globulin remaining in the ammonium sulphate solution may be extracted as a precipitate by boiling the liquid.

☞ **Quantitative Determination of Globulin.**—(1) The simplest method is by use of the Esbach tube; first determine the total proteins by the Esbach method, then saturate the urine with magnesium sulphate, which precipitates the globulin. Note the increase in volume which takes place. Filter and determine the serum-albumin in the filtrate by the Esbach process again, allowing for the difference in volume in amount of urine used. The difference between the second and first reading indicates the amount of globulin. The inaccuracy of the Esbach tube renders this method only approximate.

(2) More accurately the filtered urine is rendered neutral with ammonia and an equal volume of saturated solution of ammonium sulphate added to it. After stirring well, the mixture is allowed to stand several hours. It is then filtered through a dried and weighed filter. The precipitate of globulin, etc., on the filter is washed with a half-saturated solution of ammonium sulphate until the filtrate ceases to cloud with silver nitrate solution. The filtration and washing require much time. Finally the whole, including the funnel, is placed in the oven and dried for half an hour at 110° C. (230° F.). Hot water is then used to wash out the ammonium sulphate and the remaining precipitate washed with alcohol and ether, and finally dried again at 110° C. in the weighing glass, if necessary, until of constant weight. The amount of urine used should be such that the total weight of precipitate obtained is under 0.3 gramme.

Clinical Significance.—In diseases its significance is much the

same as that of albumin, but there are certain points of difference, the globulin being a variable factor. As a nephritis improves, the relative amount of globulin diminishes and increases with each acute exacerbation. The amount of globulin compared with albumin (in the blood 1 to 1.5) is *increased* in albuminuria due to gastrointestinal toxemia or other digestive disorders, in chronic catarrhal cystitis, pyonephrosis, acute nephritis, cantharides poisoning, and pneumonia. A significant increase is claimed in amyloid kidney and in the albuminuria of pregnancy, in subacute nephritis of the tubular variety and in diabetic albuminuria. Senator claimed that an increase of the globulin-albumin ratio is a fairly constant symptom of lardaceous disease and of some diagnostic importance.

Of the globulins separately it is claimed that euglobulin and fibrino-globulin are increased in the mildest cases of physiological and cylic albuminuria. Euglobulin is the cause of the mildest form of albuminuria.

Globulin is *decreased* compared with albumin in contracted kidney, in chronic renal hyperemia, and in the albuminuria of typhoid fever.

Clinical Note.—Since an improvement in nephritic process is usually attended by a diminution in the amount of globulin, the determination of the globulin quotient possesses a certain clinical value.

ALBUMOSES OR PROPEPTONES.

Since pepsin is always present in the urine the most commonly occurring albumoses are secondary or deutero-albumoses. Primary albumoses (hetero-albumoses) are rarely found in urine. A substance known as Bence-Jones albumose or Bence-Jones body occurs in rare instances and was formerly deemed a primary albumose, but more recently this has been questioned. Albumoses in the proteolytic sequence lie between serum-albumin and globulin, are not coagulated by heat, but are precipitated by ammonium sulphate in acid solution.

Source.—Some albumoses found in urine are probably identical with the ordinary digestive albumoses which get into the blood,

others with those which are formed at the expense of the tissue albumins under the influence of various micro-organisms, and others still with those which are strictly an expression of a metabolic abnormality in itself, as in the various non-septic fevers, phosphorus poisoning, etc., perhaps due to the action of the various tissue ferments. The amount found in urine is usually small, but that of the Bence-Jones body may be large; the latter appears to be derived from the bone-marrow.

Clinical Significance.—Unless the urine is free from albumin the occurrence of deutero-albumoses is of little significance. The albumoses are found in such a great variety of conditions as to be of little special clinical value. They are found whenever there is a breaking down of blood cells (leukemia, scurvy, etc.), in cases of increased breaking down of tissues or exudates (fever, cancer, etc.), in severe diseases of the liver, in cases of pus-absorption, gangrene, etc., etc. Some writers regard them as a valuable sign of concealed pus somewhere in the body. Gastric or intestinal ulcers are accompanied by albumosuria and in such cases somatose given internally (40-60 grammes) can be detected as albumoses in the urine. In syphilitic nephritis the amount of the albumoses is relatively large. In postcoital urine of men and when the patient is on a milk albumoses may be found.

Emerson thinks that excluding seminal fluid, milk diet, and errors in albumin testing, a marked albumosuria may be of clinical interest in cases of suspected abscesses in inaccessible parts of the body, in differentiating cerebro-spinal meningitis from tubercular meningitis, and ulcer in the stomach or intestines from other condition not cancer. Mixed albumosuria is the term given to the condition when albumosuria alternates with albuminuria.

Bence-Jones' body occurs in considerable amount in *diseases of the bones.* The condition is rare, not over 35 cases being recorded. Multiple myelomata have been the cause in all cases reported but one (lymphatic leukemia). Dr. R. H. Fitz, of Boston, has found it in myxedema, in multiple and latent tumors of the trunk, and in multiple tumors of the bones.

The largest amount of albumoses is found in bone diseases; in cases of sarcoma of the bones of the trunk the quantity may reach 0.5 per cent.

Tests for Albumoses.—☞ (1) The simplest test is that of Heller, which in the cold gives a white ring, soluble, however, on warming, and reappearing on cooling. (2) The most accurate test is the biuret, which gives a red color with the albumoses. Urobilin also responds to the same test, hence must be removed. The method of Hammarsten modified by Bangs is as follows: 10 c.c. of urine are well mixed with 8 grammes of finely powdered ammonium sulphate. The mixture is kept at the boiling point for ten seconds and centrifuged hot. The supernatant fluid is decanted off and urobilin removed from the sediment by shaking with alcohol which is poured off. The residue is then mixed with water, heated to boiling, and filtered to remove albumin. The filtrate is shaken with chloroform and allowed to settle; the aqueous supernatant fluid is then removed and tested with the biuret test which gives a violet-red color, especially on warming.

(3) Alder prefers to remove albumin, if present, by use of trichloracetic acid, adding 15 per cent. To from 6 to 10 c.c. of the urine, thus treated, if necessary and filtered are added 1 or 2 drops of hydrochloric acid and then a 5 per cent. phosphotungstic acid solution until precipitation is complete. The precipitate, after decantation, is repeatedly washed with absolute alcohol, the latter being removed each time by centrifugalizing and decanting. Finally, when all the urobilin has been thus extracted, leaving the sediment white, the latter is rinsed out with distilled water, shaken with strong sodium hydroxide solution until all blue color disappears, and then tested with the weak copper sulphate solution for albumoses, which, if present in amount 0.2 gramme per liter (0.02 per cent.), will yield the red color characteristic of albumoses.

(4) To show albumoses along with albumin acidify the urine strongly with hydrochloric acid and add an equal volume of a saturated solution of common salt. Boil, and serum-albumin is coagulated. Filter hot, and when the filtrate cools it will become cloudy if albumoses are present.

(5) The method of Hofmeister given above may be used for removing albumin and globulin, *i. e.*, addition of sodium acetate solution, enough ferric chloride to give a deep red color neutralizing nearly with dilute sodium hydroxide solution. boiling and filtering. The filtrate is tested for iron with acetic acid and potassium ferrocyanide and, if the blue color appears, the whole filtrate must be made slightly alkaline, warmed again and filtered again. The process is repeated until no iron is found in the filtrate. To 25 c.c. of the filtrate is then added 3 c.c. of strong hydrochloric acid and then phosphotungstic acid, as long as a precipitate forms. Warm the mixture on the water-bath until the precipitate settles, decant, add distilled water twice and warm again twice, then decant, dissolve the precipitate in weak solution of sodium hydroxide, heat until yellow, and then heat with a drop or two of 2 per cent. copper sulphate solution.

Tests for the Bence-Jones Body.—☞ (1) The simplest clinical test is the following: warm the urine gently; if a precipitate appears, heat to boiling. If the precipitate is dissolved, but reappears on cooling, the Bence-Jones body is present. If the urine is placed in a beaker, acidulated with acetic acid and heated cautiously, the temperature being taken with a chemical thermometer, it will be noticed that at 52° C. (96.5° F.) a slight cloud appears; at 55°, a marked turbidity; around 60°, a dense cloud; around 90°, a tough coagulum, and after 5 minutes, at 100° C. (212° F.), a clear solution, which, however, on cooling clouds again.

(2) Heat the upper portion of the filtered urine, add nitric acid, drop by drop, with shaking after each addition, and note a pinkish tint in the heated portion.

(3) Add nitric acid, drop by drop, to the cold urine. An abundant precipitate appears, which is dissolved by warming.

(4) The biuret test may be used. Saturate the boiling urine with ammonium sulphate, filter, wash the precipitate with saturated ammonium sulphate solution, dissolve in water and test with sodium hydroxide and weak copper sulphate, a violet-red color being obtained.

(5) **The Sulphide Test of Boston.**—The loosely bound sulphur in the Bence-Jones body serves for detection of it by formation of lead sulphide, when acetate of lead is added to the urine under proper circumstances. To 15 c.c. of urine are added an equal volume of a saturated solution of sodium chloride and the mixture shaken thoroughly. Alkali is then added (3 c.c. of 30 per cent. sodium hydroxide) and the mixture again thoroughly shaken. Next boil the upper third of the urine in a test-tube and add 10 per cent. lead acetate solution, drop by drop, heating after each drop. Lead sulphide is formed, which, in the form of a brown precipitate turning black, is seen after one minute.

Clinical Note.—The occurrence in the urine of a substance which causes a cloud when the urine is warmed (around 60° C., 140° F.) and which disappears on boiling to reappear on cooling, attests the presence of the Bence-Jones body. The tests 2-5, above, may be used as confirmatory.

Quantitative Determination.—The quantity of the Bence-Jones body may be determined by use of the Esbach tube or the centrifugal method. The author can give from personal experience no information as to the best method to be used.

Clinical Note.—The amount of it is usually large and albuminuria is not usually an accompaniment, while ordinary albumosuria is small in amount of albumoses present and accompanied by or alternating with true albuminuria. Occasionally, the Bence-Jones body forms a sediment in acid urine. (See **Sediments.**)

Prostatic Albumin and Albumose.—Ballenger, of Atlanta, claims that, if the urine after massage of the prostate contains an appreciable amount of albumin or albumose, it invariably means disease of the prostate or seminal vesicles. He uses the nitro-magnesian test, viz., 1 part of nitric acid to 10 parts saturated magnetism sulphate solution, the test being made by the contact method.

MUCOID AND SO-CALLED NUCLEO-ALBUMIN.

Urinary mucus occurs in normal urine in two portions, an insoluble and a soluble one. The insoluble portion forms the mucous cloud or nubecula, but the minute soluble portion can

only be extracted from the urine with chloroform after treating with acetic acid. The term *mucoid* is applied to the protein thus occurring in urinary mucus. Mucoid resembles mucin in most respects, but is soluble in excess of acetic acid. Mucin also occurs in urine, but not normally. In addition to these proteins there are others, namely, *the so-called nucleo-albumins* and true nucleo-proteins. The so-called nucleo-albumin is the substance which causes the appearance of the upper ring when Heller's cold-nitric-acid-by-contact test is applied, and also of the precipitate when acetic acid is added to urine in the cold. The so-called nucleo-albumin has been called Moerner's body, and euglobulin, and may occur normally in the urine. True nucleo-albumin never occurs normally, but in certain pathological states is claimed to be present.

Chemistry.—The mucins are secreted by the epithelium of the genito-urinary tract, are soluble in water, in concentrated solution become viscous and are converted by acetic acid into a gelatinous mass. Mucoid differs from the mucins in general, in that the precipitate from dilute solutions with acetic acid is more or less soluble in excess and the precipitate with acetic acid is not as slimy as that of mucin. Both mucin and mucoid are glyco-proteins, *i. e.*, compounds of a protein molecule, with a carbohydrate-like molecule, hence from them can be split off a reducing body. The mucoid of normal urine, according to Moerner, resembles the ovomucoid of the hen's egg. Mucins are not precipitated by boiling, and they stay in solution even after addition of acetic acid, when a sufficient amount of a neutral salt, as sodium chloride, is added to the urine.

Mucoid occurs in small amount in urine, in the nubecula mostly, but also in minute amounts in solution. The mucoid in solution can not be obtained merely by precipitation with acetic acid, but must be extracted by chloroform. The so-called nucleo-albumin, or Moerner's body, when precipitated from urine in the cold by acetic acid is not readily soluble in excess of acetic acid. According to Moerner it is a compound of serum-albumin with an acid (chondroitin-sulphuric, nucleinic, and taurocholic), but Matsumoto claims it is a compound of euglobulin and fibrinogen.

True nucleo-proteins are found in the urine as a result of destruction of cell-nuclei in the kidney or elsewhere. These substances are compounds of nuclein with protein. The nuclein in turn is a compound of a protein with nucleic acid. Nucleo-proteins contain phosphorus and when digested by pepsin yield nuclein. They yield no reducing body when treated with dilute acid.

Physiology.—As already stated the source of mucoid is the excretion from the epithelium of the genito-urinary tract and its presence is to be inferred by the appearance of the so-called mucous cloud. Small amounts of the so-called nucleo-albumin are found in normal urine, and the quantity is increased in the new-born and in adults after severe exercise.

Clinical Significance.—Mucoid is increased in all conditions in which there is a hyper-secretion of genito-urinary mucus, hence in prostatitis, cystitis, pyelitis, etc.

In rare cases true mucin appears. Four such cases are on record, analogous to mucous colitis, when long mucous plugs are found in the urine, which may be in the form of casts of the ureters and the renal pelvis.

The so-called nucleo-albumin or Moerner's body is especially increased as a forerunner of nephritis; it also is increased in essential albuminuria and may exceed the amount of serum-albumin in febrile albuminuria. It is also said to be increased in leukemia, jaundice, acute infections and fevers, in connection with albuminuria due to poisons, in acute yellow atrophy of the liver and after compression of the thorax.

☞ **Clinically,** we find it most commonly in the urine of women containing vaginal fluids, in catarrhal conditions throughout the urinary and genito-urinary tract, and in all urine containing bile.

The nucleo-proteins proper occur in conditions attended by destruction or degeneration of the epithelium of the kidneys or kidney pelvis, hence in acute nephritis or acute parenchymatous degeneration, as from poisoning, in chronic renal hyperemia, pyelitis, etc.

Clinical Tests.—(A) To demonstrate *mucoid,* dilute 25 c.c. or more of urine with equal parts of water or until it has a specific gravity around 1010, then add acetic acid, drop by drop, as long as a precipitate forms, being careful not to redissolve the latter by excess of acid. Then repeatedly filter the urine until it is clear, and wash with cold water acidulated with acetic acid. Finally, dissolve the precipitate in weak sodium hydroxide solution and collect the solution as it runs through the filter in a previously empty tube. Acidify this filtrate with 2 per cent. hydrochloric acid, boil two or three minutes, render alkaline again, and heat with a few drops of Fehling's solution, which is reduced by mucoid, but not by so-called nucleo-albumin.

(B) To demonstrate the so-called *nucleo-albumin or Moerner's body* a number of methods may be used as follows: ☞ 1. Heller's cold nitric acid test by contact shows a white zone or diffuse cloud in urines above the line of contact and the test repeated is plainer in urine diluted with 3 parts water.

2. The same urine freed from albumin and globulin by boiling, filtration, and cooling, if diluted with water as above, should show a precipitate when 50 per cent. acetic acid is added to it in the cold. The cloudiness is best seen by comparison with the diluted urine to which no acid has been added (mucoid gives the same reaction, but does not respond to Heller's test or those below). Moreover, the cloudiness in the case of so-called nucleo-albumin is insoluble in excess of acetic acid, but soluble in ammonia.

3. The urine freed as above from albumin and globulin also gives a precipitate when saturated with magnesium sulphate, and the precipitate is soluble in weak ammonia.

4. The presence of nucleo-albumin can be still further demonstrated by overlaying a concentrated solution of citric acid in the cold with filtered urine, a white cloudy ring at the line of contact indicating it. (Albumin and globulin are negative to citric acid.)

5. Ott's test is applied by adding to 5 c.c. of clear filtered urine an equal volume of a saturated solution of sodium chloride and then 1 to 2 c.c. of Almen's tannin solution, which immediately

gives a white precipitate. The tannin solution is made by dissolving 5 grammes of tannic acid in 250 c.c. of a 40 to 50 per cent. methylated spirit to which 10 c.c. of 25 per cent. acetic acid have been added.

(C) To demonstrate the true nucleo-proteins in urine is a difficult and usually impossible task, since the presence of phosphorus must be proved.

MISCELLANEOUS PROTEINS.

Fibrin occurs in the urine in the clots of hematuria. It also occurs, but more rarely, without blood. In such a case the urine coagulates spontaneously after it is voided, becoming a jelly-like mass or a shredded mass. Occasionally, the coagulation occurs within the body and shreds or chunks are passed with symptoms in some cases resembling renal colic. The *chemistry* of the coagulation is the occurrence of fibrinogen in the urine which is split into fibrin by the fibrin ferment most always present in the urine. The *clinical signficance* of fibrinuria is either hematuria, chyluria, or violent inflammation of the lower urinary passages. In the latter event it has been known to occur following the application of a cantharides plaster, in a case of renal abscess, and in severe subacute glomerular nephritis (chronic parenchymatous, so-called). In most cases of hematuria with clots the blood is renal in origin, but fibrin from the bladder is common in villous tumors of that origin.

Detection.—If the urine on exposure to the air coagulates spontaneously, fibrin is present. If it coagulates when heated slightly to, say, 56° C. (132.8° F.) and the coagulum does not dissolve on further heating, fibrin may be suspected.

Samples of urine submitted to the author for examination for fibrin usually turn out to contain mucopus and phosphates in form of lumps, plugs, or masses of cast-like form. Warm the mass or clots for several hours on the water-bath with a 1 per cent. solution of sodium carbonate, and if fibrin, it will dissolve and when filtered the solution gives a deep-red color with Millon's reagent.

Nucleo-histon and *histon* are found in the urine in leukemia and other diseases in which there is leukocyte destruction.

Nucleo-histon is rich in phosphorus and is precipitated by acetic acid in the cold.

To demonstrate it the entire 24 hours' urine is boiled and filtered to remove albumin. A large excess of 95 per cent. alcohol is added, the mixture filtered and the precipitate obtained on the filter washed with hot alcohol and dissolved in boiling water. On cooling the solution is acidified with hydrochloric acid and allowed to stand a long time. Filter to remove uric acid and precipitate with ammonia. Wash the precipitate on a small filter with weak ammonia water until the washings no longer respond to the biuret test, dissolve in dilute acetic acid and test the solution for histon, (1) by boiling and obtaining a coagulum soluble in mineral acids, and (2) by the biuret test.

SYSTEMATIC EXAMINATION OF THE URINE FOR PROTEINS.

J. E. Dale devises the following scheme of analysis for the detection of urinary proteins: any examination for protein bodies presupposes chemically pure acetic acid and clear urine; if the urine is turbid and the matter in suspension passes through the filter paper, it will be well to mix with the urine a small quantity of magnesium carbonate in fine powder, allow the mixture to stand for a few minutes, and then filter. (1) A portion of the clear urine in a test-tube is acidified with acetic acid; a clouding indicates *nucleo-albumin*. If a precipitate forms, it should be filtered. (2) A. A portion of the filtrate of No. 1 (or if this be negative, the clear acidified urine) is added slowly to a portion of a saturated solution of common salt, a precipitate may be any of the following—*any albumose (except deutero-albumose), histon or globin*. B. If a precipitate is not formed, the addition of urine is continued until it is in excess of the salt solution; the upper third is shaken and boiled; a clouding indicates *serum-albumin*. C. If a precipitate is formed, it should be filtered, care being taken that the urine has not been added beyond the point at which it is saturated by the salt solution. The filtrate should be boiled. A clouding indicates *serum-albumin*. D. If a posi-

tive reaction is had in A, a portion of the original urine is saturated, first, with saturated salt solution without adding acetic acid, to determine whether a precipitate is formed in neutral solution, and, second, to determine whether the body present is one of which loosely combined sulphur is a characteristic, using Boston's method. (3) A few drops of the original urine are added, a drop at a time, to a considerable quantity of clear water. Milky streaks in the track of the drops indicate *globulin.* (4) A. A portion of the original urine is acidified with acetic acid and filtered; the filtrate is then rendered faintly alkaline with ammonium hydrate and boiled for a few minutes, then filtered. The filtrate may contain *peptone* or *deutero-albumose.* B. The second filtrate from A is saturated with ammonium sulphate and boiled. A white precipitate indicates *deutero-albumose* (yellow or brownish, ammonium urate). C. B is filtered and filtrate examined for *peptone;* if deutero-albumose has been found, the saturation with ammonium sulphate must be complete and the boiling decided to assure its separation.

It will be noticed in the above that Dale adheres to the word nucleo-albumin instead of using the term Moerner's body.

CHAPTER XX.

Pathological pigments: hemoglobin and derivatives; biliary pigments; melanin, etc.
Hemoglobinuria and hematuria.
Hemoglobinuria:—causes and significance.
Paroxysmal hemoglobinuria:—conclusions of C. H. K. Macalister.
Clinical tests for hemoglobin: the benzidine test; the guaiacum test; Heller's test; Donogany's test.
Teichmann's hemin test.
Spectroscopic test for hemoglobin; methemoglobin and oxyhemoglobin.
Hematuria; causes; how to distinguish renal blood in urine.
Clinical significance of hematuria.
"Essential" hematuria.
Hematoporphyrinuria; clinical significance; relation to sulphonal poisoning, anemia, cirrhosis, etc.
Clinical tests and quantitative determination of hematoporphyrin.
Melanin:—appearance of urine containing it; clinical significance; relation to neoplasms.
Clinical tests for melanin:—ferric chloride test of von Jaksch; Zeller's test; the nitroprussiate test.
Differentiation of melanin from alkapton.
Biliary pigments and acids:—choluria.
Appearance of the urine in choluria.
Clinical significance of choluria.
Clinical tests:—Gmelin's, Rosenbach's, Huppert's, Trousseau's; advantages and disadvantages of them.
Modifications of Huppert's test.
Miscellaneous tests for bile pigment:—Hammarsten's; Bouma's; shaking with chloroform.
Removal of the bile from urine.
The bile acids: clinical significance and tests; Hay's sulphur test; A. Jolles's test; Udranzsky's test; Platner's crystals; Oliver's peptone test.

The coloring matters which are of pathologic import in the urine are hemoglobin and derivatives, the biliary pigments, and melanin; certain other pigments are claimed to be present in certain pathological states.

HEMOGLOBIN AND DERIVATIVES.

This is the red pigment of the blood which contains iron and is in constitution a protein. It occurs in the urine in two conditions known as *hemoglobinuria* and *hematuria* respectively. Hemoglobinuria is the term used for the condition in which the blood pigments pass directly into the urine without the corpuscles; hematuria is the term for the condition in which both appear. The coloring matters of the blood found in urine are hemoglobin, oxyhemoglobin, methemoglobin and hematin.

Hemoglobinuria.—Whenever, for any reason, the liver is unable to transform into bilirubin all the blood-coloring matter set free by destruction of red corpuscles, hemoglobin appears in the urine. This occurs when about one-sixtieth of the total hemoglobin is set free.

☞ Urine containing hemoglobin with but few or no corpuscles is usually acid in reaction, contains but little albumin, and may deposit a copious rusty-colored amorphous sediment, while the supernatant urine above it may be clear and of normal color, though in most cases the clear urine is blood-colored, and if nephritis be associated with it the urine is likely to be more or less cloudy.

Clinical Significance.—1. Hemoglobinuria is most frequently observed after poisoning by potassium chlorate, arsenetted hydrogen, sulphuretted hydrogen, creasote, pyrogallic acid, naphthol, hydrochloric acid, tincture of iodine, carbolic acid, carbon monoxide, phosphorus, and also by morels (Helvella esculenta, certain mushrooms).

2. Hemoglobinuria may occur in the course of any one of the specific infectious diseases, as scarlatina, icterus gravis, variola hemorrhagica, yellow fever, typhus, and probably syphilis.

Its occurrence in malaria is disputed, while malarial hematuria is well known. The author saw a case of hemoglobinuria following infection from an injury to the kidney.

3. Hemoglobinuria follows injection into the blood of solvents of the corpuscles, as glycerine, solutions of bile-salts, or distilled water; also after transfusion of the blood of animals into man.

4. It occurs in pyemia, scurvy, fat-embolism, some cases of jaundice, after extensive burns, occasionally in Raynaud's disease, and in leukemia complicated by icterus.

It may occur during pregnancy, in certain cases of nephritis, and after severe intra-abdominal hemorrhages. ☞ The author saw a case of it in the scurvy of an infant on an artificial food.

5. From unknown causes as an epidemic among new-born.

6. In the so-called *paroxysmal hemoglobinuria* the attacks are frequently preceded by chills and fever closely simulating malarial fever. It must be, however, distinguished from malarial *hematuria*. Simon and others doubt the existence of malarial hemoglobinuria, while malarial hematuria is well known.

In paroxysmal hemoglobinuria the immediate causes are usually excessive exercise or mental excitement, cold plunges, etc. It lasts for a period of one or two days or less. There may be pain in lumbar region with chills and fever preceding it.

The urine in paroxysmal hemoglobinuria is red or dark brown and the spectroscope shows methemoglobin alone or together with hemoglobin; the microscope shows masses or casts of amorphous pigment and crystals of hematoidin; tube-casts and renal epithelium may be present, as also oxalate crystals. The urine always contains albumin and sometimes bile pigment.

The disorder occurs only in predisposed subjects and may follow trivial causes; it may appear to be hereditary, and may run in families. Occasionally it alternates with cyclic albuminuria. Such slight agencies as tying a string around the finger, or changing the posture, may induce an attack, which may also follow slight blows, psychic shocks, etc. The etiology is unknown, as in the case of the epidemic hemoglobinuria of children. Paroxysmal hemoglobinuria has recently been studied by C. H. K. Macalister:—paroxysmal hemoglobinuria is the result of intravascular hemolysis. The intravascular hemolysis occurs when the blood serum becomes autolytic. The latter is most frequently brought about by the application of external cold. Variation of the severity of the paroxysm may depend upon variation of the excitability of the nerve centers. Paroxysmal hemoglobinuria and Raynaud's

disease are closely related. Syphilis is a possible antecedent in many cases. There are no gross changes in the abdominal viscera. There is possibly a passive hyperemia of the kidneys, but no inflammatory change.

Clinical Tests for Hemoglobin.—1. The most delicate test for hemoglobin is the **benzidine** *test,* as follows:—to 10 c.c. of urine add about 1 c.c. or less of glacial acetic acid and shake well. Then add 3 to 4 c.c. of ether and shake well. Let stand a few minutes and add 5 to 10 drops of absolute alcohol. Shake gently, remove the ethereal layer with a pipette and add it to a fresh solution of 0.5 of Merck's pure benzidine in sufficient glacial acid to dissolve it and to which 2 or 3 c.c. of a 3 per cent. hydrogen dioxide solution have been added. The mixture should then be well shaken and a green or blue color is seen, according to whether the amount of hemoglobin is small or large.

This test is not interfered with by the various substances which render other tests uncertain, as *e. g.,* pus, bile, and other accidental constituents.

☞ 2. **Guaiacum Test.**—When for any reason the benzidine test can not be used the next most available procedure is to try the *guaiacum test.* Prepare the reagents as follows:—make a fresh alcoholic solution of guaiacum resin, 1:5 in strength; let it stand a few minutes and filter. Take commercial oil of turpentine which has been exposed to the air until it thickens and dilute it with five volumes of ordinary turpentine. Mix equal parts of the fresh guaiac solution with the turpentine, shake well, until an emulsion is formed, add urine in equal volume to the emulsion, shake well, add a few drops of alcohol and let it stand, when a blue or bluish-green color slowly forms, if blood is present. The test may also be performed by the contact method, when the blue ring will be seen at the juncture of the emulsion and the urine. Warming the mixture does not affect the blue ring if due to blood, but a blue ring due to pus is dissolved by heat. This test is more readily made when an ordinary commercial solution of hydrogen dioxide is substituted for the ozonized turpentine, which requires time to prepare.

The test will not succeed in alkaline urine, which must be acidified with acetic acid, and freshly voided urine is preferable in all cases.

Another way of performing the guaiac test is as follows: to 10 cc. of urine in a large test-tube add twice as much ether and mix well by pouring from one tube to another several times. Add a few grains of powdered guaiacum and mix well again. Then add 5 cc. of glacial acetic acid and mix again. Let settle, pour off the supernatant liquid, and divide equally between two test-tubes; set one aside for control and to the other add 2 cc. fresh hydrogen dioxide, using a pipette and carrying it to the bottom. A bluish discoloration anywhere shows blood to be present.

3. **Heller's Test.**—This is the simplest test of all and can be used in cases when it is difficult to obtain chemical reagents:—to a test-tube half full of urine add five drops of a 30 per cent. sodium hydroxide solution, shake well, and warm. The earthy phosphates are precipitated and form a brownish-red or blood-red precipitate due to mixture of blood-pigments with the earthy matters. If the urine is alkaline, add one-fourth its volume of normal urine before applying the test. If the urine is dark colored, so that the red color of the phosphates can not be seen, filter, and dissolve the precipitate in acetic acid; a red solution is obtained which gradually is decolorized on exposure to the air. Hematoporphyrin interferes with this test, and also certain drugs.

4. **Donogany's test** is said to detect 1 part of blood in 8000 of urine. To 10 c.c. of urine add 1 c.c. of ammonium sulphide solution and 1 c.c. of pyridin, when in the presence of hemoglobin an orange-red color appears, seen best by looking through the tube lengthwise.

5. **Teichmann's Hemin Test.**—In this test crystals of hematin hydrochlorate are obtained and identified by the microscope. The phosphatic precipitate obtained in Heller's test, or better a precipitate obtained by adding tannic acid, is thrown on a filter, washed, and allowed to dry in the air. A very small granule of this dried precipitate is put on a glass slide with a similar granule of common salt and a few drops of acetic acid. A cover glass is

put on and the slide allowed to stand for 24 hours, at the end of which time the microscope will show the characteristic crystals of hemin. If time is valuable, the crystals may be obtained by cautious warming over a small flame with constant renewal of acetic acid, which should only steam, not boil, and when brownish in color should be no longer heated. The slide should not be cooled suddenly, but very slowly.

6. **Spectroscopic Test.**—Render the urine feebly acid with acetic acid and place before the open slit of a spectroscope in a test-tube, beaker, or small trough with glass sides, when the two bands of oxyhemoglobin (arterial blood) will be seen, either at once or upon carefully diluting with distilled water so that the latter floats upon the urine. Add ammonium sulphide and note spectrum on venous blood (reduced hemoglobin). The bands of oxyhemoglobin are noticed between the lines D and E; the line of reduced hemoglobin is single between D and E; the methemoglobin bands are four in number with a dark band in the red between C and D, if the reaction of the urine is acid. In most cases of hemoglobinuria the spectrum of methemoglobin is obtained. (Fig. 24.)

In order to be sure that the spectrum of methemoglobin is obtained both that of the neutral and of the alkaline urine must be obtained, the latter by adding ammonia to the urine. Bile and urobilin may cause confusion, and if these bodies are present it is best to precipitate the urine with basic lead acetate, and examine the filtrate, which contains hemoglobin only, without methemoglobin. If no bands are seen, add ammonium sulphide and obtain reduced hemoglobin.

Clinical Note.—Preceding the onset of nephritis after scarlet fever minute amounts of hemoglobin may be found in the urine.

☞ **Hematuria.**—In this condition we find not only hemoglobin chemically, but the microscope shows red blood corpuscles also. The condition is due to hemorrhages into or within the urinary or genitourinary tract. The urine is cloudy, if the blood is present in appreciable amount. The color ranges, according to the amount of blood, from light smoky to bright red if hemoglobin

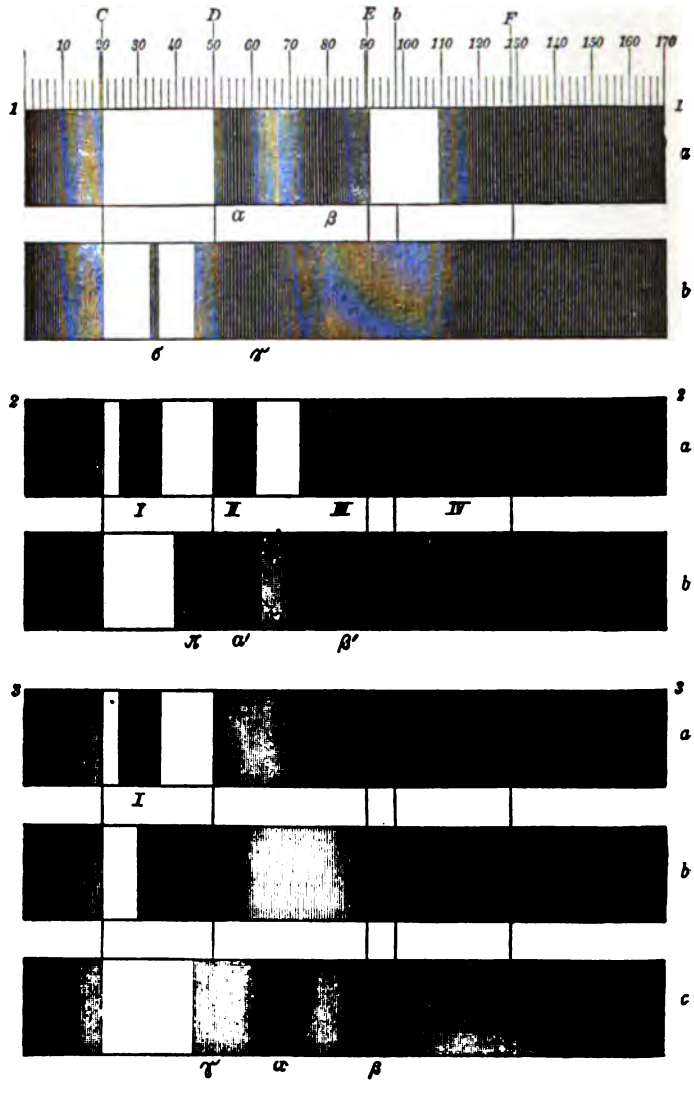


FIG. 24.—Spectra: 1, *a*, Oxyhemoglobin; *b*, oxygen-free hemoglobin. 2, Methemoglobin: *a'*, in neutral solution; *b'*, in alkaline solution. 3, Hematin in acid alcoholic solution; *b*, in ammoniacal solution; *c*, reduced hematin (after Neubauer and Vogel).

is present, or dark brownish red if due to methemoglobin, as in capillary hemorrhages. To distinguish renal blood in the urine the following points are of service: the urine and the blood are well mixed so that if the urine is voided into two glasses the same amount of blood appears in each; clots are not common, but, if present, may be large, as in cancer of the kidney; washing out the bladder the washings finally come away clear; tube-casts, if present, suggest renal origin of the blood; if tube casts are absent, the microscope reveals presence of *blood shadows* in the freshly voided acid urine; phenomena of micturition, (pain, frequency, straining), if absent, point to a renal origin for the blood.

Clinical Significance of Hematuria.—The significance of hematuria is that of hemorrhage into or within the urinary tract which may be due either to (1) blood diseases and malignant forms of infections, (2) kidney diseases, (3) diseases of the lower urinary passages, (4) injuries to the urinary tract, (5) essential without known cause, (6) of menstrual origin in women or (7) due to fistulous openings into the urinary tract.

Among general diseases hematuria is most common in malignant forms of acute specific fevers, as small-pox, malaria, and typhoid, but it also occurs occasionally in leukemia, and in diseases of the blood:—hemophilia, scurvy, purpura, etc.

☞ In kidney diseases blood is most commonly found in *acute disturbances,* especially severe acute hyperemia and acute nephritis. Hematuria from such conditions is common in poisoning by turpentine, carbolic acid, formaldehyde preparations and cantharides. In subacute nephritis a hemorrhagic form occurs, but the amount of blood is not usually so great as in acute exacerbations. Hematuria may occasionally occur in chronic interstitial nephritis as a result of vascular changes, and occasionally it may be found in chronic renal hyperemia. It may occur in amyloid kidney as a result of amyloid infiltration about the smaller blood vessels, but is uncommon or at least slight.

☞ Renal hematuria is common in tuberculosis, cancer and calculus of the kidney; in cancer it may be profuse with large clots in the urine; in tuberculosis it occurs at the onset, especially

when the papillæ are involved, and when pus is usually abundant: the patient rises at night to void bloody urine; in calculus the blood is increased by physical exercise, strain, lifting, etc., but not always immediately thereafter, and the day urine contains more blood than the night or after repose. Hematuria is fairly common in cystic kidney, and occurs commonly in parasitic diseases of the organ, in renal infarctions and in congestion due to venous thrombosis, as in the new-born; renal embolism, purpura hemorrhagica, hydatids, and abscesses also cause renal hematuria. Renal varix is a condition, difficult to determine, which sometimes causes hematuria.

Hematuria due to diseases of the lower urinary tract is found in stone, tumors, ulcers, parasites, injuries, and varicose conditions. Papilloma of the bladder, vesical calculus, gonorrheal conditions and prostatic diseases are clinically common causes.

Injuries to the kidneys are frequently followed by hematuria, as also when other portions of the urinary tract suffer. Operations upon and explorations of the genitourinary organs are a common cause of hematuria.

Essential hematuria is the term applied to a rare condition of middle adult life in which no known lesion can be found, but nervous causes are suspected. The cases are likely to occur in women and may recover spontaneously, after treatment of the nervous system, after operation on the kidney, or even after simple exposure of the organ.

Blood may be mixed with the urine of menstruating women or be due to uterine or intestinal hemorrhages, either with or without fistulous openings. In all cases in which blood is found in the urine of women the vagina, as a source, must be excluded.

Hematoporphyrinuria.—Hematoporphyrin, $C_{16}H_{18}N_2O_3$, is a pigment derived only from the blood and present in traces in normal urine; it is identical with iron-free hematin. Urine containing appreciable amounts of this substance is reddish-brown in a thin layer, but by reflected light may be opaque or almost black. In some cases it is cherry-red, Bordeaux red, or of port wine color. The exact source and mode of formation of the pigments are not

known. According to Garrod and others the condition in cases not due to sulphonal is the result of perverted catabolism of hemoglobin, but nothing definite has been proved.

☞ **Clinical Significance of Hematoporphyrinuria.**—The long continued use of sulphonal, trional, or tetronal causes hematoporphyrinuria, as well as acute poisoning by these drugs. It is also increased in acute infectious diseases (typhoid), tubercular lesions, rheumatism, pericarditis, Addison's disease, paroxysmal hemoglobinuria, cirrhosis of the liver, pneumonia, hematemesis, Graves' disease, primary anemias, gout, and lead poisoning.

Clinical Note.—Where hematoporphyrin is found in increased amount in urine in cases not acute infections we should think first of poisoning by sulphonal or similar drugs; second, of functional disorders of the ductless glands; third, of primary anemia; fourth, of cirrhosis of the liver or of gout, and lastly, of lead poisoning.

Clinical Tests for Hematoporphyrin.—Urine of a wine-red color is typical of hematoporphyrinuria. On standing it may become darker, almost black. Spectroscopic identification is necessary and for this purpose it must be isolated as follows:—The urine is precipitated with barium mixture (containing one part of saturated barium nitrate solution and two parts of concentrated baryta water) until no further precipitate forms on the addition of the reagent. The sediment now contains the bulk of the hematoporphyrin, together with any other pigments that may be present; the precipitate is filtered off, washed, and extracted with dilute (3 per cent.) hydrochloric acid alcohol. This extract should be reddish or pink in color, should fluoresce and should turn dark on heating. The most characteristic feature is the spectroscopic appearance (Fig. 25), hematoporphyrin in acid alcoholic solution showing a narrow absorption band in the yellow between C and D, and a second broader band between the yellow and the green, between D and E. If the solution is now rendered alkaline, four bands will appear, one between C and D, two between D and E, and a fourth very dark band between C and F, *i. e.*, between the green and the blue.

Quantitative Estimation of Hematoporphyrin.—100 cc. of urine

are rendered alkaline with a few drops of a 10 per cent. sodium hydroxide solution, treated with a few drops of a 10 per cent. solution of calcium chloride until no further precipitate forms, the reaction of the liquid being kept alkaline by the addition of sodium hydroxide solution. The dark red precipitate is filtered off, repeatedly washed with water until the washings are free from chlorides, then washed with absolute alcohol to get rid of

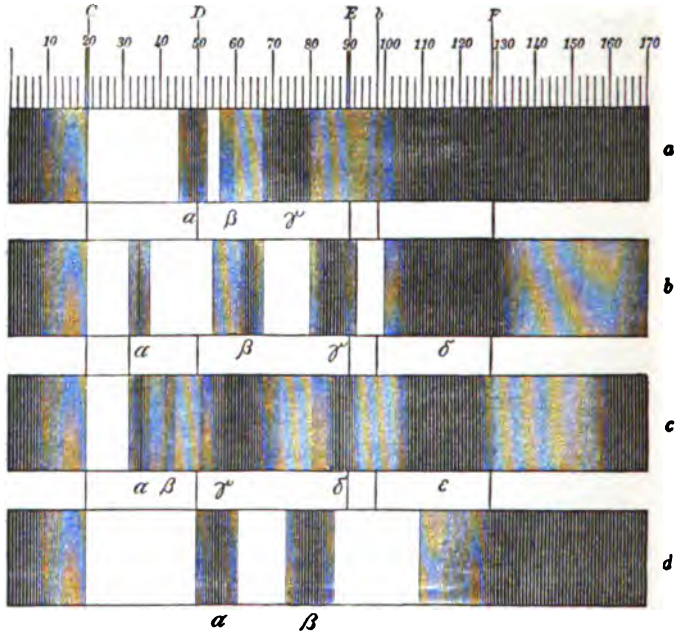


FIG. 25.—Spectrum of Hematoporphyrin: *a*, Acid; *b*, alkaline; *c*, neutral; *d*, metallic spectrum (Saxe).

the water, and, finally, extracted with dilute alcohol, acidulated with hydrochloric acid, at a temperature of about 40° C. (104° F.). From the pink alcoholic extract the pigment is precipitated with water after the liquid is first carefully neutralized with dilute ammonia. The flocculent precipitate is gathered on a filter, washed free from chlorides, the water removed with alcohol, the alcohol with ether, the residue dried at 115° C. (239° F.) to a

constant weight and the weight determined. A weighed portion of the total is incinerated and the amount of ash determined. From this the entire amount of inorganic ash is calculated and subtracted from the total weight determined, the difference representing the hematoporphyrin.

MELANIN.

A pigment occurring in the urine in some cases of malignant growths and other conditions is known as melanin. The urine is normal in color when voided, since the chromogen of melanin is colorless. On exposure to the air this chromogen splits and forms melanin, which gives the urine a dark hue. The development of the dark color may be hastened by addition of nitric acid or other oxidizing agents. Like alkapton, it causes a darkening of the urine from above downward and the same strikingly defined layer above the colorless urine may be observed. Small black particles of melanin may be found in the sediment, appearing under the microscope as amorphous scales.

Rarely the urine is black when voided.

Clinical Significance.—The condition is a rare one and most commonly signifies a melanotic sarcoma somewhere in the body. But it is often absent in cases of such growths and may be present in long continued malarial fevers and in severe wasting diseases. It is important to differentiate it from alkapton, which causes a similar darkening of the urine, and also from urobilin and indican. The presence of it may be deemed valuable corroborative evidence of the melanotic character of a neoplasm, as *e. g.*, of the skin or the eye.

Clinical Tests for Melanin.—(1) The test of von Jacksch with ferric chloride is as follows: add to the urine a few drops of a fairly concentrated solution of ferric chloride, when a gray color and precipitate of phosphates carrying the gray color is seen; excess of the iron solution redissolves the gray color (alkapton similarly treated gives a disappearing dark-green color with grayish phosphatic precipitate). This is said to be the only delicate and reliable test. Dilute solution of neutral ferric chloride is said to produce a brown or black color.

(2) Zeller's test: bromine water gives a yellow precipitate which gradually blackens. (In carboluria the yellow is permanent when bromine is added.)

(3) The test for creatinine with sodium nitroprussiate, caustic alkali and acetic acid gives a Prussian-blue color.

The chromogen may be isolated by precipitation with baryta water and purifying.

☞ **Clinical Note.**—To differentiate from alkapton it is well to take the clinical history into consideration, since melanin occurs in pathological states and alkapton in physiological ones. Deposit of black pigment in the cartilaginous parts of the body, as *e. g.*, in the ears, suggests alkaptonuria, while a growth or wasting disease suggests melanuria.

THE BILIARY PIGMENTS AND ACIDS.

The condition when bile pigments are present in the urine is known as *choluria*. Of the different bile pigments bilirubin is the only one occurring in freshly voided urine, the others forming as the urine stands. The pigments which occur in choluria are bilirubin, biliverdin, bilifuscin, biliprasin, cholecyanin, and choletelin, all oxidation products of bilirubin.

☞ **Choluria.**—Urine containing bile-pigments is peculiar for its color, odor, foam, and the color of its sediment. The color is from bright yellow (traces) to greenish-brown or almost black when bile is abundant. In some cases all the pigment adheres to a urate sediment in the urine. The odor suggests ox-gall. The foam is yellow. The urine stains filter paper yellow. The sediment is stained yellow. The microscope shows epithelium and casts to be stained with an intense golden yellow color. Heller's cold nitric acid test for albumin shows the so-called "nucleo-albumin" ring in marked degree, and the acid also produces a marked color zone.

Clinical Significance of Choluria.—☞ Jaundice ☜ is responsible for most cases of choluria, but it also is known in severe infectious diseases, in phosphorus poisoning, and in any condition in which there is destruction of the red-blood corpuscles. The bile pigments may appear in the urine several days before

icterus is perceptible, and they may occur in diseases of the liver without jaundice. The diseases in which bile is most seen in the urine are, besides catarrhal jaundice, biliary calculus, parasites, compression of the duct by tumors of the liver, of the gall-bladder, the duct itself, and of neighboring structures, namely, the pancreas, stomach, and omentum; in diseases in which the blood pressure in the liver is lowered; in cases in which degenerative processes are affecting the glandular epithelium, as in acute yellow atrophy, or where the destruction of red corpuscles is going on so rapidly that the liver cannot transform into bilirubin all the blood pigment carried to it, as in pernicious anemia, malarial intoxication, typhoid fever, poisoning with arsenetted hydrogen, etc.

In severe cases of jaundice, etc., in addition to bile pigments, bile acids are also present. (See below.)

Obermayer and Popper claim that by means of the iodine test and a modification of Bouma's test bile pigments may always be found demonstrated in normal urine, and the amount of bile-pigment varies in accordance with the intensity of the urinary color. An increase occurs in cardiac diseases or myocardial affections with hyperemic manifestations, and in atrophic cirrhosis of the liver. Also in certain febrile diseases:—croupous pneumonia, acute articular rheumatism, and a group of pleuritic exudates.

Clinical Tests for Bile Pigment.—These are often unsatisfactory unless strictly fresh urine is obtained, and for other reasons. The classical tests are Gmelin's, Rosenbach's, Huppert's and Trousseau's or Maréchal's.

☞ 1. **Gmelin's Test.**—In this test the urine is superimposed upon nitroso-nitric acid by the contact method: if bilirubin is present, layers of colors will be seen in the urine which from above downward are green, blue, violet, red, and yellow. The green color is necessary for the test, since the other colors may be due to indican or urobilin. The *disadvantages* of this test are many:—if the nitric acid contains too much or too little nitrous acid, the green color may fail to be seen; the nitric acid should

be made faintly yellow by adding to it a small piece of wood and boiling. Remove the wood and cool the acid. The urine in most cases should be diluted to 1005, and fresh urine is desirable. Urine rich in indican may give a deceptive green (blue with yellow). If there is much albumin in the urine the colors will be hidden by the white ring, and removing the albumin takes the bile with it. Antipyrin interferes with the test.

The test may be made by mixing the urine with a concentrated solution of sodium nitrate and by floating the mixture upon sulphuric acid; or a crystal of sodium nitrate may be dropped into strong sulphuric acid and the urine floated upon this.

2. **Rosenbach's Test** is to filter much urine previously acidulated with hydrochloric acid through a white filter paper, partially dry the paper by pressure with dry filter paper and add one drop of the yellow nitric acid. Rings of color are seen as above, the green being external. The same test may be applied by use of a porous porcelain plate. When the amount of bile is very small, the urine may be shaken with chloroform in proportion of 4 of urine to 1 of chloroform. Let settle, decant, and pour the chloroform upon the filter.

Rosenbach's test can be more rapidly made as follows: moisten a piece of white filter paper with the urine, place it on a porcelain surface, and let a drop or two of yellow nitric acid fall upon the centre of the wet paper. In the presence of bile-pigment concentric rings of blue, violet, green, and yellow will appear, but a red color alone is not positive.

3. **Huppert's Test,** useful when the urine is rich in indican or dark in color, is as follows:—10 c.c. of urine are made alkaline with sodium carbonate and calcium chloride solution added as long as a precipitate occurs. The filter with the precipitate is then placed in a porcelain dish and alcohol containing 5 per cent. of hydrochloric acid is added and the whole heated on the water bath. In the presence of bile a green to a blue colored solution is obtained which, on cooling, turns blue, violet, and red on addition of nitric acid. The test may also be performed as follows: 10 to 20 c.c. of urine are precipitated with milk of lime, or a solu-

tion of barium chloride, and the precipitate, after filtering, brought into a beaker by perforating the filter and washing its contents into the latter with a small amount of alcohol acidulated with sulphuric acid. The mixture is boiled, when, in presence of bilirubin, the solution assumes an emerald green color. Urine tested for bile should be freshly voided.

Another modification of Huppert's test is that of Nakayama: to 5 c.c. of acid urine is added an equal amount of 10 per cent. barium chloride solution and the mixture centrifugalized. The supernatant clear fluid is poured off and 2 c.c. of the reagent fluid added to the precipitate. (The fluid consists of ferric chloride in proportion sufficient to form 4 grammes per liter in a mixture of 99 parts of 95 per cent. alcohol and 1 part strong hydrochloric acid.) Heating the mixture of precipitate and reagent fluid gives a green or bluish green turned violet or red by yellow nitric acid.

Steensma further modifies the original Huppert test by adding one drop of a 0.5 per cent. solution of sodium nitrite to the acid alcohol mixture before warming on the water-bath.

4. **The Iodine Test.**—This is usually known as Maréchal's test, but Emerson thinks Trousseau's name should have priority: —the urine acidified with acetic acid is either mixed with tincture of iodine or a 1 per cent. solution of iodine in alcohol is floated upon the urine. A green color appears at the line of contact, if bile be present. If the urine be mixed with the iodine, the green color is all through the mixture. The test can be used in urines rich in indican, but it is said to react with normal urines occasionally.

5. **Miscellaneous Tests.**—A great number of tests for bile have been proposed: certain aniline dyes give peculiar colors with biliary urine: thus a solution of *azure-blue* causes a green coloration, and one of *methyl violet* a red. Hammarsten uses an *acid mixture* of 1 part 25 per cent. nitric acid and 19 parts of a 25 per cent. hydrochloric acid allowed to stand until yellow. To 1 part of this 4 parts of alcohol are added, each time when a test is made, 10 c.c. of urine are placed in a 15 c.c. centrifugal tube and a few c.c. of barium chloride solution added. The mixture is centrifugal-

ized for a minute, the supernatant fluid poured off, 1.2 c.c. of the acid mixture added to the sediment, the mixture shaken well, and centrifugalized half a minute. A green solution is obtained, if bile is present.

Bouma's Test, which can be made a quantitative one, is to add to 10 c.c. of fresh urine 2 c.c. of a 20 per cent. calcium chloride solution and to neutralize nearly with ammonia. The slightly acid urine is centrifugalized, the sediment washed, the fluid decanted, and 5 c.c. of a mixture of 4 c.c. of absolute alcohol and 1 c.c. of Obermayer's reagent are added, all poured into a test-tube and compared with a set of six standard tubes to match the biliverdin formed. (For Obermayer's reagent he uses a solution of 1.5 grammes of ferric chloride in 1 liter of hydrochloric acid of a specific gravity 1.19.)

Clinical Note.—The presence of bile in the urine in amount more than the merest trace can be inferred by the physical characteristics and especially by the staining of epithelium, casts, etc., in the sediment seen with the microscope. Chemical tests are likely to be unsatisfactory, but the author prefers the centrifugal methods described above. Shaking biliary urine with chloroform is a simple and sometimes successful test if the urine be previously accidulated with hydrochloric acid, the bile imparting a yellow color to the chloroform.

Removal of Bile from Urine.—Acidify the urine with hydrochloric acid and shake the bile out with chloroform.

THE BILE ACIDS.

It will be convenient to consider these bodies in this chapter. They occur in two groups, glycocholic containing nitrogen but no sulphur, and taurocholic containing both nitrogen and sulphur. They occur only in pathological conditions and are found in severe forms of liver trouble, as in hepatic congestion, cirrhosis, tumors, and severe acute catarrhal jaundice. They also occur in anemia, hemoglobinuria, scurvy, and splenic leukemia, less commonly in amyloid infiltration of the liver.

Clinical Tests.—☞ (1) The simplest test is Hay's sulphur

test. On the surface of urine which does not exceed 17° C. (63° F.) in temperature at the highest is sprinkled a little finely powdered sulphur. If the sulphur sinks at once, a 1 :10000 content of bile acids is indicated; if it sinks after shaking gently and waiting one minute, 1 :40000. A little of the sulphur will remain on top of the urine and coalesce to form a thin granular pellicle. In case of doubt compare the behavior of sulphur with normal urine in another beaker. The author has seen this test work well in cases where bile-pigment was undoubtedly present in urine. The test depends upon a disturbance of surface tension. The author has noticed that sulphur sinks also in urine containing methylene-blue or its chromogen.

2. **A. Jolles' Test.**—Fifty c.c. of urine are mixed with 15 c.c. casein solution (3%); the casein is then precipitated by adding from 0.6—0.8 c.c. sulphuric acid (10%). The precipitate is placed on a filter and acted upon by 10 c.c. absolute alcohol at room temperature. Four to five c.c. of this alcoholic extract together with one drop of a 5% rhamnose solution and 4-5 c.c. concentrated hydrochloric acid are heated to a weak boil for one or two minutes and, on being cold, shaken with 2 c.c. ether. In the presence of bile acids a green fluorescence will ensue. Taurocholate of sodium can be demonstrated by this method when present in dilution of 0.05%. Urines rich in indican and aromatic oxyacids should not be tested by this method.

3. **Udránzky's Test.**—One cubic centimeter of the urine is treated with one drop of a one-tenth per cent. solution of furfurol and slowly superposed upon 1 cc. of concentrated sulphuric acid, care being taken by immersing the tube in cold water to prevent too great heating of the mixture. A purple color appears at the plane of contact, that gradually extends upward into the superposed solution; on standing, the color turns bluish. In alcoholic solution a green fluorescence is seen. The pigment gives a typical spectrum; viz., two bands, the one near F, the other between D and E, near E.

4. **Platner's Crystals.**—An attempt may be made to obtain crystals of the sodium salts of the bile-acids by evaporating the

solution with sodium hydroxide, extracting the residue with absolute alcohol, and adding enough ether to produce a slight clouding. On standing, Platner's crystals of the sodium salts of the bile-acids form. These may further be identified as above or by testing their effect on the heart of a curarized frog (retardation).

5. **Oliver's Peptone Test**—To 20 minims of clear filtered urine reduced to 1,008 in specific gravity add 60 minims of test-fluid prepared as follows:

Pulverized peptone, ...gr. xxx.
Salicylic acid, ...gr. iv.
Acetic acid (B. P.), ...m. xxx.
Distilled water to .....fl. oz. viii.

(Solution to be filtered repeatedly until transparent.)

If bile salts are present in quantity greater than normal, a distinct milkiness promptly appears, becoming more intense in a moment or so. If the bile salts are normal or less than normal there is no immediate turbidity, but in a short time a slight tinge of milkiness is seen.

CHAPTER XXI.

THE SACCHARIDES (SUGARS) IN URINE.

Saccharides, sugars, and other carbohydrates in urine.
Definition of term "carbohydrate."
Chemistry of the saccharides occurring in urine.
Monosaccharides, disaccharides, and polysaccharides; aldoses and ketoses.
Properties of carbohydrates as a class.
Properties of monosaccharides.
Properties of disaccharides; meaning of inversion.
Properties of polysaccharides.
"Sugar" in the urine:—glucose (dextrose).
Other sugars occurring in the urine.
Chemistry of glucose:—properties and reactions.
Physiology of glucose: role of the liver; source of the sugar in the blood; sugar in normal urine.
Pathology of glucose: alimentary glycosuria; "assimilation limit;" test of the "limit;" toxic, experimental, psychic and secondary glycosuria.
Diabetes mellitus: quantity of sugar in the urine; degrees of the disease.
Detection of sugar in the urine: collection of the specimen; kind of person subject to glycosuria; early symptoms of diabetes mellitus.
Physical tests for sugar.
Chemical tests: tests of Moore or Heller, and of Rubner.
Reduction tests for sugar: Trommer's test; technique; advantages and disadvantages; principle of the test.
Fehling's test; how to make Fehling's solution; criticism of authorities; technique of the test; criticism of authorities.
Advantages and disadvantages of Fehling's test.
Haines' test with a permanent solution: advantages and disadvantages.
Benedict's test.
Nylander's test with bismuth subnitrate: technique; principle; advantages and disadvantages.
The Boettger-Bruecke tests: solutions and technique.
Miscellaneous tests: permanganate; Penzoldt's, Johnson's, etc.
The picric acid test.
Detection of sugar in normal urine.

Microchemical tests: the Neumann-Thierfelder method with phenylhydrazine; C. E. Simon's method; Williamson's test.
Fermentation tests; Einhorn's saccharimeter.
Fermentation and Nylander's test combined.
Polariscope test: half-shadow saccharimeters.

The term carbohydrate, formerly given to important constituents of plants, was intended to signify that these bodies contain carbon together with oxygen and hydrogen, the latter in the same atomic proportion as in water, *i. e.*, two of hydrogen to one of oxygen. But inasmuch as some of them have been found in which the hydrogen and oxygen do not conform to this proportion, and inasmuch as this proportion occurs in bodies not carbohydrates, the term *saccharide* may be used to designate them with greater propriety.

In order to understand the occurrence and reactions of the saccharides in urine it is necessary to review the chemistry of them.

Chemistry.—The group $C_6H_{10}O_5$ is known as the saccharide group, and saccharides are classified according to the number of these groups which they contain; thus monosaccharides, containing one molecule $C_6H_{10}O_5$ only, disaccharides containing two, and polysaccharides, containing an unknown number. Monosaccharides and disaccharides contain in addition one molecule each of water. The monosaccharides (glucose) are subdivided into trioses, pentoses, hexoses, etc., according to the number of oxygen atoms they contain, but the pentoses have only five atoms of carbon, thus $C_5H_{10}O_5$, and lack the molecule of water.

The monosaccharides found in urine are glucose (dextrose) levulose and galactose, all having the formula $C_6H_{10}O_5$. $H_2O = C_6H_{12}O_6$, hence are *hexoses*. In addition there are *pentoses*, of which animal gum is probably one.

The disaccharides (saccharoses) found in urine are maltose, lactose, isomaltose, and sucrose, all of the formula $(C_6H_{10}O_5)_2 + H_2O = C_{12}H_{22}O_{11}$. The polysaccharides (amyloses) found in urine are starch, dextrine and glycogen, of the formula $(C_6H_{10}O_5)x$. These compounds, chemically speaking, are alde-

hyde or ketone derivatives of complex alcohols, and considered with reference to their derivation are termed *aldoses* and *ketoses* respectively. Aldoses contain the alcohol group CH_2OH and the aldehyde group COH, while ketoses contain the alcohol group and the ketone group CO. Glucose (dextrose) is an aldose, but levulose is a ketose, both being hexoses.

Carbohydrates or saccharides, as a class, are either fermentable or can, in the majority of cases, be converted into fermentable substances. They are neutral and non-volatile, decomposed by heating to a suitable temperature. Most of them are in the solid state, white in color, and soluble in water. Such as are soluble possess the property of rotating the plane of polarized light. They have a more or less sweetish taste and many, especially glucose, are *strong reducing agents,* taking away oxygen in alkaline solutions from higher oxides of the heavy metals, and converting them into lower oxides or into the metallic state.

Oxidizing agents convert the saccharides into saccharic and mucic acids, finally into oxalic acid. The carbohydrates occur in great quantity in vegetables, familiar examples being cellulose, starch, and sugar. In the animal kingdom they are found in milk, honey, blood, urine, etc.

The monosaccharides (simple sugars, glucoses) can not be split up into two or more simpler sugars. They contain from 3 to 9 atoms of carbon, usually the same number of oxygen atoms and double the number of hydrogen atoms. They are white, odorless, sweet, neutral, crystallizable bodies, readily soluble in water, especially in hot water, slightly soluble in alcohol, but more so in hot alcohol, and insoluble in ether. The trioses, hexoses, and nonoses undergo alcoholic fermentation, and most of the group are optically active. They resemble the aldehydes and ketones in the property of being readily oxidized, hence are strong reducing agents, many tests for "sugar" in the urine depending on this property.

In addition solutions of them acidified with acetic acid possess the characteristic property of yielding with phenylhydrazine crystalline precipitates of yellow insoluble substances known as

osazones. The monosaccharides in urine are dextrose, levulose, and galactose.

The disaccharides (sucrose, lactose, maltose) resemble the monosaccharides physically and in their solubility, but differ by not directly undergoing alcoholic fermentation and by not reacting with phenylhydrazine. Moreover, cane-sugar does not reduce cupric oxide in alkaline solution, hence the inability of medical students to obtain the "sugar test" by adding cane-sugar to urine. The disaccharides may be split up into simpler sugars by hydrolysis, *i. e.*, by taking up a molecule of water under treatment by dilute mineral acids, or by the action of enzymes; thus, sucrose splits up into dextrose and levulose or undergoes **inversion** as the term is, since sucrose rotates the plane of polarized light to the right, but the mixture of dextrose and levulose to the left through the influence of levulose, which turns the plane more to the left than dextrose does to the right.

The polysaccharides (amyloses) comprise the starches, gums, cellulose, etc., and are either almost insoluble in water or wholly so, and do not crystallize nor diffuse through animal membranes. Hydrolysis splits them into dextrines, disaccharides and monosaccharides.

SUGAR IN THE URINE.

The so-called sugar in the urine is really glucose (dextrose). Other sugars also occurring are levulose, galactose, lactose, isomaltose, maltose and pentoses. In addition such carbohydrates as animal gum, starch, glycogen, and dextrine occasionally have been found. Of these by far the most important clinically is **glucose.** Normally, glucose, animal gum and maltose are found in minute amounts; sometimes also pentoses. The total of the carbohydrates, normally present, measured as glucose and including allied bodies, glycuronates, chondroitin-sulphuric acid, mucoid and nucleinic acid, is stated by Emerson to be from 2 to 2.23 grammes in 24 hours. Lactose occurs in conditions which can not always be deemed pathological. The other sugars occur chiefly in diabetes.

Glucose (dextrose, grape-sugar) is present in minute amount,

from 0.38 to 0.62 grammes per 24 hours, and not revealed by the ordinary clinical tests in normal urine.

Chemical Constitution of Glucose.—$C_6H_{12}O_6$, a carbohydrate containing 6 atoms of carbon, is one of the class of *monosaccharides,* group *hexoses,* sub-group *aldoses.*

Reactions and Properties:

1. Absorbs oxygen when heated with strong alkaline solutions, giving rise to characteristic color and odor.

2. Heated with alkaline solution of cupric salts, reduces them with (red) precipitate of cupric oxide.

3. Reduces bismuth subnitrate to the metallic condition, when heated with it in the presence of an alkaline solution.

4. Warmed with a solution of phenylhydrazine hydrochloride in water, to which a little sodium acetate is added, forms a yellow crystalline precipitate of phenylglucosazone.

☞ 5. Fermented by yeast splits into alcohol, carbon dioxide, and a number of other substances.

6. Boiled in faintly alkaline solution colored blue by indigo exhibits a beautiful color reaction.

7. Gives color reactions with various substances, as with alphanaphthol in presence of sulphuric acid. (Molisch's reaction.)

8. Gives a deep red with picrates in alkaline solution.

Glucose is soluble in its own weight of water, hence occurs only in solution in the urine and is not found microscopically. Filtering the urine is of no avail in removing it, therefore, and not a necessary performance in the execution of tests for it. An important property of glucose has been stated above, namely, its strong reducing power, such that when heated with an alkaline solution of certain metallic salts (copper and bismuth) it reduces them, *i. e.*, removes oxygen.

Another important reaction is its formation with excess of phenylhydrazine of an osazone, phenylglucosazone (phenyldextrosazone), a yellow insoluble compound which crystallizes in characteristic groups of yellow needles of definite melting point.

Glucose is only slightly soluble in alcohol, more so in hot alcohol, insoluble in ether. From its solutions it may be extracted by

use of animal charcoal. It crystallizes in colorless, transparent prisms, which collect in bundles or in hard tenacious crusts. Solutions of it turn the rays of polarized light to the **right,** hence the name **dextrose.** The specific rotation of the aqueous solution, according to Tollens, is 52.50°.

Oxidation converts it first into monobasic gluconic acid, $C_6H_{12}O_7$, then into dibasic saccharic acid, $C_6H_{10}O_8$, and finally into acids of lower molecular weight.

Physiology.—Glucose exists in normal blood in minute amount (0.1 to 0.15 per cent.) and appears in the urine when this amount exceeds 0.3 of 1 per cent., the excretion by the urine preventing a further increase in the blood. Normally, the liver transforms glucose into glycogen, but if an undue amount of glucose reaches it, the quantity in the blood increases and finally it appears in the urine. The ability of the liver to transform glucose into glycogen is much less than that of the intestinal epithelium to transform the disaccharides and polysaccharides into glucose, and this ability varies in different individuals.

The source of the sugar in the blood is first the carbohydrates in the blood, and, second, the proteins and fats of the tissues. After the polysaccharides have been split up and the cane-sugar inverted, they are for the most part absorbed into the portal circulation by which they reach the liver where the greater amount is stored up in the form of glycogen, a less amount passing into the blood beyond. The body tries hard to keep the percentage of sugar in the blood up to a certain point and, if it decreases, new sugar is turned into it from the glycogen warehouses in the liver, muscles, glands, and elsewhere, or, in case of deficit, sugar is formed from proteins and probably also from fats. But if the sugar is increased in the blood, while at the same time the glycogen warehouses are filled, then the body gets rid of the superfluous amount by way of the urine. The increase in the amount of sugar in the blood (hyperglycemia) may be due either to excessive ingestion of carbohydrates, inefficient destruction of them in the blood, or by either sudden emptying of the glycogen warehouses or inability of them to store the sugar brought to them.

According to B. Schoendorff *(Archiv. f. d. gesammte Physiologic,* CXXI, p. 572), the method of Patein and Dufau shows from 0.0105 to 0.0274 per cent. of sugar in every normal urine. Excessive ingestion of carbohydrates may increase it to 0.1 per cent.

Pathology.—Glycosuria, *i. e.,* the occurrence of sugar in the urine in amount sufficient to respond to the ordinary clinical tests, is due to a number of causes as follows: (1) **alimentary glycosuria,** due to an excess of carbohydrates in the food, commonly when the amount of sugar ingested at one time is about 200 grammes, although the amount differs according to the individual, in some persons the ingestion of 250 grammes failing to produce glycosuria. If a person have glycosuria from ingestion of so small amount as 100 grammes (about 3 ounces) of glucose, the condition is to be regarded as pathologic, showing a diminished power of utilizing carbohydrates in the system (carbohydrate intolerance). It is thought by some observers that this glycosuria is due to an hepatic insufficiency which, in turn, is referable to a mild form of diabetes mellitus, other conditions being excluded.

The term **assimilation limit** is used to designate the minimum amount of sugar, ingestion of which by the mouth is followed by appearance in the urine. This limit is lowered by a number of conditions, as hunger, pregnancy, hepatic cirrhosis, faulty nutrition, fatty degeneration of the liver, phosphorus poisoning, diffuse cerebral lesions referable to alcohol and syphilis, lead colic, exophthalmic goitre, functional neuroses, as hysteria and any condition in which there is diuresis. Hence, in order to test satisfactorily the assimilation limit of the individual, the above conditions must be excluded. Moreover, no matter how much starchy food is taken no sugar should normally be found in the urine, hence the finding of such sugar after a diet rich in starches, but free from sugars, is, when the conditions above mentioned can be excluded, extremely significant of the existence of diabetes mellitus.

☞ **Test of the Assimilation Limit.**—This is done by the

method of Naunyn as follows: a cup of milk and coffee together with a slice of bread are taken for breakfast, two hours after which the 100 grammes of dextrose are also taken. If any sugar appreciable by clinical tests is found in the urine afterwards, the assimilation limit is lowered; if as much as 1 per cent., the case is likely to be diabetes mellitus. The excretion of sugar begins in about an hour after the dextrose is taken, is at its height in from 2 to 4 hours, and lasts only 8 to 10 hours.

2. **Toxic Glycosuria.**—Glycosuria may be due to the action of drugs and poisons: curare, carbon monoxide, amyl nitrite, morphine, ether, strychnine, cocaine, and mercuric chloride.

The narcotics appear to asphyxiate the epithelial cells of the body and, hence, to interfere with the normal oxidation of the sugar. Oxygen starvation from any cause, as in suffocation or death agony, may produce glycosuria. A special form of glycosuria is that produced by administration of *phloridzin*. In this case no increase in the amount of sugar in the blood occurs, the glycosuria being entirely a renal one.

Extracts from several of the ductless glands, as from the thyroid and suprarenal capsules, injected into the blood may cause glycosuria.

Transfusion of the normal salt solution and also injection of sugar into the blood may cause glycosuria. Administration of caffeine, theobromine and other diuretics may cause it, but without hyperglycemia.

3. **Experimental and Traumatic.**—Sugar is found in the urine after certain experiments on the brain, and in certain cerebrospinal diseases due to formation of an excessive amount of glucose in the body at the expense of glycogen, etc.

Removal of the pancreas may be followed by a high degree of glycosuria. Injuries to the liver and central nervous system in experiments on animals are followed by the appearance of sugar, especially injuries to the floor of the fourth ventricle and severe injuries to the skull. Removal of the thyroid has been followed by the appearance of sugar in the urine. Fracture of the cervical vertebræ is also a cause. Traumatic neuroses, as shock from railroad accident, are said to produce it.

4. Psychic.—Following mental strain or worry, and fatigue with anxiety, probably due to nervous or vasomotor disturbances which affect glycogen formation in the liver and muscles, or which accelerate or retard the combustion of sugar.

5. Secondary to various diseases, especially those of the intestinal tract or of the nervous system: apoplexy, brain tumors, and hemorrhages at the base of the brain: tetanus, cerebral and spinal meningitis, paralytic dementia, tabes, multiple scleroses, diseases of the sympathetic nervous system, functional neuroses, exophthalmic goitre, gout, arteriosclerosis, obesity, paresis, new growth in the pancreas, occlusion by calculus of the pancreatic duct with atrophy of the gland, acromegaly, syphilitic lesions of the nervous system, liver, or pancreas; following apopletic, epileptic and hystero-epileptic attacks; renal hemorrhages, chyluria, and occasionally nephritis, especially just before death from chronic nephritis (edema of the brain); acute infections, particularly during convalescence (typhoid, scarlet fever, measles, cholera, diphtheria, la grippe, and especially malaria, due possibly to irritation of the floor of the fourth ventricle by toxines). Sugar is also said to be found in the urine in the course of pregnancy; (care should, however, be taken to differentiate glucose in the urine from lactose, which also responds to some of the so-called sugar tests). Richartz reports a case of glycosuria seeming to have arisen on the basis of a catarrhal inflammation of the intestinal mucosa.

In most of these cases, however, the occurrence of sugar is **transitory** except those in connection with brain lesions, particularly those affecting the floor of the fourth ventricle, and in most cases also the amount of sugar is small or soon becomes small.

☞ **6. Diabetes Mellitus.**—Most of the cases of persistent glycosuria which we encounter in practice are due to this malady, in some cases of which it is claimed that atrophy of the pancreas or degeneration limited to the islands of Langerhans is found post-mortem. In diabetes mellitus of average severity the per cent. of sugar is from 4 to 6 according to the patient's diet, the higher figure when fruit is eaten freely. In extreme cases 10 per cent.

of sugar may be found. On a strict diet excluding carbohydrates the per cent. of sugar drops to 1 or less; on some days sugar may not be found at all by the usual clinical tests.

☞ **Degrees of Diabetic Glycosuria.**—Diabetes mellitus is either mild, medium or severe in character. Mild cases are those in which withdrawal of carbohydrates from the food is immediately followed by disappearance of the sugar from the urine; medium, those in which to remove the sugar it is necessary not only to stop the carbohydrates, but to reduce the proteins in the diet until in adults less than 18 grammes of nitrogen (36 grammes of urea), but more than 10 (20 of urea), or in children less than 13 and more than 7 are found in the 24 hours' urine; and severe those in which the withdrawal of carbohydrates must be accompanied by the reduction of proteins to a point where less than 10 grammes of nitrogen in adults and 7 in children are found in the urine. *In the most severe cases, absolute withdrawal of all food fails to remove the sugar completely from the urine.*

The three degrees are accounted for on the theory that in the mildest cases there is merely hepatic insufficiency and in the severe forms a fault of metabolism of muscle tissue, *i. e.*, the latter has lost the power of decomposing sugar which reaches it from the liver. The absence from the blood of a glycolytic ferment or corresponding kinase is also thought to have to do with the severe cases, as well as the failure of the pancreas to supply it.

☞ **Clinically,** we find the acetone bodies absent in the milder cases; present at times or in small quantities in the medium cases, and more or less persistently present and in larger quantity in the severe unmanageable cases.

The beginning of diabetes mellitus may be difficult to recognize. According to Loeb the following is true:

1. Little is known with respect to the earliest stage of diabetes.

☞ 2. The temporary occurrence of a small quantity of sugar in the urine ought not to be regarded lightly; severe diabetes sometimes follows.

3. Some cases of diabetes are acute from the first.

4. Some cases of slight and temporary diabetes recover completely.

☞5. In a great number of cases of diabetes, before a large quantity of sugar is excreted, small quantities are excreted temporarily, often for years.

Fluctuations in the quantity of sugar occur in diabetes mellitus. According to Simon the following is true:

1. Cases have been known in which 360 grammes (5,580 grains, or about one pound) of sugar in 24 hours have been passed.

2. The severity of the pathological process cannot be measured by the amount of sugar eliminated. The total amount of sugar may not exceed a few grammes daily, and yet the disease rapidly tends toward a fatal termination.

3. Absence of sugar from the urine in one or even more urinary examinations does not exclude diabetes. In such a case give the patient 100 grammes of glucose, and test the urine three or four hours after.

4. A light case of diabetes in which the sugar has disappeared under dietetic treatment may suddenly become severe, and apparently severe cases may suddenly assume a benign type.

5. In a type described by Hirschfeld a specific gravity of 1012 and greatly diminished elimination of solids is noticed.

6. Lusk speaks of rapidly fatal cases of diabetes mellitus in which no increase in ammonia nor in acetone bodies is noted, but in which 3.6 grammes of sugar are excreted for every gramme of nitrogen taken as food, showing complete inability of the body to oxidize the sugar.

☞ **Clinical Note.**—In the writer's experience the urine of all persons should be tested in the afternoon, about two hours following the noon day meal. Traces of sugar discoverable by Haines' test may be present at that time, but absent at other hours of the day.

In the writer's experience polyuria is not a constant symptom among well-cared for Americans with diabetes. Not over 50 per cent. of 70 cases seen by the writer had noteworthy polyuria, and

in his private practice the largest amount ever collected and accurately measured was 18 pints. The mortality among all the polyuric cases seen in 7 years was 42 per cent., but no typical case of glycosuria without polyuria and other marked symptoms proved fatal in that time. Half of these patients voided 20 to 40 grammes of urea per 24 hours. The prognosis was directly proportioned to the quantity of urea, the safest excretion being 20 to 30 grammes. The mortality in those voiding over 60 grammes of urea was very great.

The author has had several cases in which the patient, although intelligent, was not aware of having any disease, even when there was considerable polyuria and over 64 grammes of sugar daily.

The thirst is greatest in cases where the percentage of sugar rises above 4 per cent. In a case in which there was 6 per cent. of sugar thirst was intense, but when, under the writer's treatment, the sugar fell to 4 per cent., the patient declared that he drank no more water than was prescribed for him and was no more thirsty than usual.

DETECTION OF SUGAR IN THE URINE.

It is important to detect the presence of sugar, even in small quantities, in urine, since in a great number of cases of diabetes before a large quantity appears small quantities may be found by the expert from time to time. Regulation of the diet and habits of the individual may be followed in such cases by satisfactory results. By such measures the author appears to have prevented the development of true diabetes mellitus in several instances. Not infrequently, however, a large amount of sugar abruptly appears.

☞ **Collection of the Specimen.**—In suspicious cases the urine voided two hours after the noonday meal is the urine to test for sugar. Considerable quantities (as much as 2 per cent.) may then be present and be absent or much diminished at other times in the day. Traces of sugar may be present at that time and wholly absent at other times of the day. It is well for the person being examined to eat the usual American carbohydrate lunch, *i. e.*, a "bakery lunch" on such an occasion.

In cases where it is claimed that sugar has been found and when the usual tests of the urine of all micturitions for the 24 hours fail to show the presence of sugar, the assimilation limit test of Naunyn (see above) may be tried. The grape sugar to be used can be had of E. H. Sargent & Co., or the commercial syrup obtained of confectioners. Cheap candy also answers for the test. "High livers" should have their urine tested after drinking champagne or other sweet wine. The author found sugar in the urine of one patient after eating bananas, but not when they were omitted from the dietary.

Middle-aged persons who are growing fat should be watched for the appearance of sugar. Also nervous, petulant, quick-tempered men or women in whose family history hysteria, epilepsy, syphilis, alcoholism, gout or rheumatism occur. Children who have to urinate often and who drink a good deal should be suspected of diabetes and any person who rises often at night should be examined for the presence of sugar in the urine. **An unusual thirst and the voiding of much urine** always suggests the possibility of diabetes mellitus.

TESTS FOR SUGAR.

The various tests may be classified as follows: physical, chemical, microchemical and spectroscopic.

Physical Tests.—The simplest test is by tasting the urine, a method occasionally practiced by clinical patients. The amount of sugar must be considerable, one would think, to overcome the natural salty taste of the fluid. Another simple test is the "boot-black's" test: a few drops of urine are allowed to evaporate upon a well-polished leather shoe. If much sugar is present in the urine, a white spot is seen on the shoe. Or some of the urine is allowed to dry on a towel or other cloth which stiffens, if much sugar is present. Or a drop or two of urine is allowed to dry in a white dish. When dry it is heated to about 200° C. (392° F.) and a yellowish brown substance appears having an odor of caramel.

The specific gravity of urine shows the presence of sugar in some cases. Any urine three pints or more in volume per 24

hours of a specific gravity of 1040 must necessarily contain sugar and any urine of such volume with a specific gravity of 1030 or more should certainly be tested for sugar.

Sugar may be obtained in quantity from the urine by evaporating the entire 24 hours' volume over the water-bath and extracting the residue with cold 95 per cent. alcohol. Any very considerable whitish residue insoluble in alcohol is likely to be sugar, and, if so, is soluble in water, especially in hot water.

Chemical Tests.—These are of two principal kinds, reduction tests and fermentation tests. In addition there are several others the nature of which is not understood.

1. Moore's or Heller's Test.—This is one of the oldest tests and is the most convenient rough chemical test: to 10 c.c. of urine add 2 to 3 cc. of liquor potassæ, mix well, hold the tube by the lower closed portion and boil the upper portion as in testing for albumin. If sugar is present, oxygen is absorbed and the color of the heated portion slowly changes to yellow, orange, and finally dark-brown. A control test with normal urine shows the difference in color. The solution of potassium hydroxide used is the ordinary U. S. P. liquor potassæ containing 5 per cent. of potassium hydroxide in distilled water. Exact strength is not necessary for the test and sodium hydroxide answers the purpose fully as well.

In performing this test a caramel odor may be obtained. The nature of the colored body formed is not definitely known; it is possibly glucinic or melasinic acid.

An advantage of the test is the fact that the chemicals used may be readily obtained, the solution is readily made, and does not deteriorate with age. The principal disadvantage is that the test is not sufficiently delicate to detect fractions of 1 per cent. of sugar in such a way as to be definite, since all urines turn more or less yellow when heated with an alkali and especially if concentrated. At least 0.3 per cent. of sugar must be present for a positive result and bile must be excluded. To make up a large amount of the test reagent dissolve ½ pound (225 grammes) of potassium or sodium hydroxide in sticks in a gal-

lon of distilled water (3850 c.c., or nearly). Any marked case of diabetes mellitus can be detected by use of this test, which was used for years by Dr. Ultzmann in his Vienna clinic. It is said that albuminous urines darken with this test and that darkening occurs when the alkali contains lead or silicates as impurity.

A modification of Moore's test, known as Rubner's test, involving the use of lead acetate and ammonia, is described by numerous authorities, but as the author has been unable to apply it successfully according to the directions given in the standard works, and as there are tests enough without it, a description of it here is omitted. Ogden classifies it among those tests most of which are greatly inferior to the copper and bismuth tests. Lactose as well as glucose reacts with Rubner's test.

3. The Reduction Tests.—These are the popular sugar tests in the hospitals and clinics. The principle of them all is the reduction by glucose of higher metallic oxides in solution either to lower ones or to the metallic state, which is favored by heat and the presence of an alkali, and results in the formation of precipitates more or less characteristic and striking to the eye. It should never be forgotten in using these tests (1) that the sugar itself does not occur as a precipitate, (2) that a large number of substances besides glucose possess the property of reducing metallic salts, hence the opportunity for error in the hands of the inexperienced is great, and (3) that certain constituents of urine possess the property of hindering the reduction. In the author's opinion more errors are made in testing for sugar than for albumin, which is saying a good deal. Moreover, the statements and directions in so-called standard works are sometimes contradictory and confusing to the operator. Reduction tests involve the use either of cupric salts or of bismuth compounds. The latter possess the advantage of not being reduced by uric acid, creatinin, and alkapton, but on the other hand are affected by constituents which are neutral to the copper tests, hence the two kinds of tests may be used one to verify the other.

The copper tests most commonly used are Trommer's, Fehling's and Haines'. In addition there are modifications of Fehling's test, such as Benedict's test.

The bismuth tests are those of Nylander-Almén and Boettger-Bruecke.

More than one method of applying the same test frequently exists and the operator is advised to practice repeatedly some one method until entirely familiar with its action on normal urine, urine containing glucose, and urine containing substances which interfere with the test.

1. Trommer's Test.—This is the original of the reduction tests and many teachers insist that their students shall become familiar with it, although, clinically, it is seldom used.

Method 1. To a test-tube half full of urine add about one-third its volume of 10 per cent. alkali (sodium or potassium hydroxide), mix well, and add further a 10 per cent. solution of cupric sulphate, drop by drop, shaking after each drop until a few flakes of the precipitated cupric hydroxide remain on slightly shaking. The upper part of the tube is now heated and, if glucose be present, a **yellow or red precipitate** appears. As soon as this happens cease heating, whereupon the precipitate will spread from above downward throughout the fluid, cuprous hydroxide (yellow) or cuprous oxide (red) being seen. It should be noted that the **precipitate** is the important point in this test and that a color without a precipitate is not positive. To control presence of other reducing substances prepare a second tube as described above, let stand without heating for 12 to 24 hours, when, if glucose be present, the precipitation of red cuprous oxide will take place in the cold.

Criticism of Authorities.—The directions in the various books for performing Trommer's test vary greatly both as to the strength of the solutions used and as to the proportions to be used. Thus we find in several books no mention at all of the strength of alkali; in others the strength varies from 10 to 30 per cent.; as to the proportion of the alkali to be used, this varies according to different authorities from one-fourth the volume of urine up to equal parts.

Regarding the sulphate of copper solution, different writers recommend from "a very dilute solution," whatever that means,

up to a concentrated one, 1:5 solution. The methods, (I and II given above), are those of Emerson and W. Simon respectively, two of our most reliable authorities. The proportions claimed to be best by Emerson are 1 of glucose, 5 of cupric hydroxide, and 11 of sodium hydroxide.

The principle of Trommer's test is that of the reduction tests in general, *i. e.*, the aldehyde or ketone structure of certain sugars gives them the property of reducing alkaline solutions of certain metallic oxides, notably of copper and bismuth. Trommer's test is a copper test and in such tests addition of an alkali to a cupric solution produces a whitish-blue precipitate of cupric hydroxide $Cu(OH)_2$, which on boiling is converted into insoluble black cupric oxide, CuO; but when a reducing sugar like glucose is present instead of the black cupric oxide, yellow cuprous hydroxide $Cu_2(OH)_2$ is formed by reduction and, on further heating, red or brownish-red cuprous oxide, Cu_2O. When no sugar is present $Cu(OH)_2$ (whitish-blue) $=$ CuO (black) $+$ H_2O. When a reducing sugar is present $2Cu(OH)_2 = 2CuOH + H_2O + O$, and on further heating $2CuOH = Cu_2O + H_2O$.

In the proportions used in Trommer's test the alkali causes a precipitate of cupric hydroxide in combination with the sugar, forming a blue solution on shaking, but in the absence of sugar only a greenish precipitation, which, on shaking, gives a cloudy green mixture. If the fluid is now heated, the cupric hydroxide is reduced by the glucose before the boiling point is reached, and yellow cuprous hydroxide or red cuprous oxide appears as a precipitate. Earthy phosphates are also precipitated in this test by the alkali used, but appear as dirty-white flocks and should not be mistaken for sugar.

The advantage of the test is that it indicates in a rough way the amount of sugar present from the amount of cupric sulphate solution necessary to add for a copious precipitation. If, for example, the undiluted urine barely responds to the test, about one-quarter of 1 per cent. of sugar may be assumed.

Method II. Some authorities prefer to add the cupric solution to the urine before adding the alkali: in this case, however,

it will be necessary to add more urine in case too much copper has been added: to about 6 or 8 c.c. of urine in a test-tube add from 2 to 4 drops of a 5 per cent. solution of cupric sulphate and then an amount of alkali equal to that of the urine; a blue or greenish transparent liquid is formed (the cupric hydroxide being dissolved in the excess of alkali) with whitish flocks of precipitated phosphates. The entire liquid is now heated, when, if sugar be present, the yellow and red precipitate forms as above.

Disadvantages of Trommer's Test.—These, as the school-boy would say, are "almost too numerous to mention," yet this criticism should apply strictly to the viewpoint of the inexperienced, since there are those clinicians who appear to use the test with much advantage and it has many strong friends. If too much copper solution is used, black cupric oxide is formed, which is not charactertistic and which may hide a small quantity of the yellow or red precipitate. If too strong alkali is used, when the urine is rich in glucose the same dark color is produced as in Moore's test above, which, mixed with the yellow or red precipitate, produces an effect which may puzzle the inexperienced. A change of color, merely, without a precipitate does not indicate the presence of sugar.

If the mixture is boiled before the precipitate appears, the latter may be due to other reducing substances than glucose as, *e. g.*, uric acid, creatinine. In such a case normally, the solution turns a dirty yellow and the precipitated phosphates are slightly tinged with yellow; if uric acid, etc., are in abnormal amounts, boiling may cause a definite precipitate as in the case of sugar.

Trommer's test fails to show glucose in amounts less than 0.2 per cent., but a **clear, brilliant, yellow color** is strongly suggestive even if the precipitate is absent.

Normal urine may give a yellow color, but it is not so brilliant as where a little sugar is present.

Since uric acid, creatinine, ammonia salts, and albumin possess the power to hold the cuprous precipitate in solution to a much greater degree than glucose is able to reduce, it is advised that

all urines be diluted in the proportion of 1:5 before the test is made, if small quantities of sugar are to be sought for, since in this dilution uric acid, etc., are too small in amount to hold the precipitate in solution, while at the same time the reducing power of the glucose is not appreciably affected.

The test, to be positive, involves decolorization of the fluid when the precipitate forms and before the boiling point is reached. A precipitate occurring after prolonged boiling or on cooling is not necessarily due to glucose, as explained above. In such a case dilute the urine as above and, if a precipitate is obtained before the boiling point is reached, glucose is present.

Ammoniacal urines sometimes cause a precipitate; too much sodium hydroxide will dissolve the cuprous precipitate, as will also strong ammoniacal urine. Too much sodium hydroxide and too much copper will give a precipitate in some samples of normal urine.

When but little sugar is present, the presence of albumin may hold the cuprous precipitate in solution, hence it is advisable to remove albumin by boiling until flocculent, cooling, and filtration.

A number of reducing substances in the urine may cause the precipitate: not only uric acid and creatinine in excess, but also hippuric acid, allantoin, mucin, hypoxanthin, urates, bile pigment, normal urine pigment, pyrocatechin, hydrochinon, the alkapton bodies or at least homogentisic acid, urobilin, and perhaps indican.

The great objection to Trommer's test and to all copper tests is the production of a precipitate, due (1) either to an increase in the amount of glycuronic acid compounds, to the presence in the urine of various drugs or to the presence of other sugars than glucose (lactose, pentoses, etc.), or (2) to the hindrance of the reaction by various constituents of the urine.

The drugs which, taken internally, cause an increase in the glycuronic acid compounds are chloral hydrate, chloroform, morphine, camphor, phenol, resorcin, thymol and menthol. A positive reduction is also obtained after use by the patient of a large number of drugs, as phenacetin, acetanilid, salicylic acid (aspirin, oil of wintergreen, etc.), benzoic acid, benzosol, chryso-

phanic acid, salol, sulphonal, antifebrin, copaiba, and other resinous drugs, turpentine, urethan, oxalic acid, rhubarb, glycerin, arbutin. Saccharin is said to hinder the reduction. In cases of poisoning by acids, alkalies, and arsenic, the urine is said to give a positive reaction with Trommer's test. Formalin, chloroform and chloral hydrate added to the urine as preservatives cause a reduction, hence the author uses boric acid for a preservative.

By way of summary it may be said that the trouble with Trommer's test is that unless the mixture is boiled the test is not sufficiently delicate, and if it is boiled other substances than sugar may yield the reaction, the golden mean consisting in momentary boiling only. In suspicious cases dilution of the urine should be practiced before deciding that sugar is absent.

2. **Fehling's Test.**—Since glucose alone cannot dissolve nearly as much cupric hydroxide as it can reduce, other tests than Trommer's have been devised in which the solution of the cupric hydroxide is aided by the addition to the test liquid of substances having the property of dissolving this hydroxide, as, for example, the alkaline tartrates, ammonia, or glycerine. Moreover, since one part of sugar can reduce about five parts of cupric hydroxide, test liquids have been devised by use of which this proportion can be obtained in practice without the possibility of the formation of the undesirable black precipitate of cupric oxide described above.

Fehling's solution is made from two liquids each of which when kept by itself is permanent. In order that there may be a maximum amount of copper in solution, *i. e.*, 5 of cupric hydroxide to 1 of glucose, Rochelle salt is used in the alkaline solution, which dissolves the cupric hydroxide as fast as it is formed on heating the cupric salt with alkali, thus keeping the solution clear and allowing the glucose to exert its reducing power on the maximum amount of hydroxide.

The other solution is merely one of cupric sulphate. Before the test is performed, equal parts of the two solutions are mixed, boiled, and the urine added instead of adding to the urine as in Trommer's test the cupric sulphate and alkali successively.

How to Make Fehling's Solution.—In answer to the question

which in the past has been asked by the State Boards of Health "How would you make Fehling's solution?" it may be said that the U. S. Pharmacopeia for 1905 and Merck's Index for 1907 describe the preparation as follows: "34.67 grammes of carefully selected small crystals of pure cupric sulphate showing no efflorescence nor adhering moisture are dissolved in sufficient distilled water to make 500 c.c., at a temperature of 25° C. (77° F.). This solution is to be kept in small well-stoppered bottles. Next a solution is made of 173 grammes of potassium sodium tartrate (Rochelle salt, U. S. P.) and 75 grammes of potassium hydroxide (U. S. P.) in distilled water sufficient to make 500 c.c. at 25° C. The solution should be kept in small rubber-stoppered bottles. When ready for use, mix exactly equal parts of the cupric solution and of the alkaline tartrate. This solution, made by mixing, keeps for a day or two only, hence should be made up fresh after that period has elapsed. Each c.c. of the mixed solutions corresponds to 5 milligrammes of glucose, *i. e.*, 0.005 gramme.

Care should be taken to notice that the directions for making Fehling's solution differ widely according to the authority.

Laboratory Note.—However it may be made, the purest obtainable cupric sulphate should be used, Merck's reagent preferred, and it is advised for quantitative purposes that the salt be dissolved in water and recrystallized. The salt should be such that when heated to 100-110° C. (212°-230° F.) it should lose 28.87 per cent. of its weight.

Technique of Fehling's Test.—Several different methods of applying the test have been proposed, of which the following appears to be the best:—Mix precisely equal parts of the copper solution and the alkaline tartrate solution and measure 5 c.c. of the clear blue mixture into a test-tube and boil. Then add not more than 2.5 c.c. of the urine to be tested and boil again not longer than 30 seconds. Or the urine can be added little at a time with momentary boiling after each addition. The same yellow or red precipitate occurs when glucose is present as in Trommer's test, but the test is more delicate, indicating, it is claimed, 0.08 per cent. of glucose, although this is disputed by Benedict. If

the urine is added drop by drop instead of all at once, an idea of the quantity present can be had, a copious yellow or red precipitate with a few drops of urine indicating a large amount of sugar. Error from reducing substances in normal urine can be avoided by diluting the urine to a specific gravity of 1005, or the mixture of reagents may be diluted before applying the test with four times its volume of water, mixed well, and the test performed by boiling the upper portion only and adding the urine drop by drop with boiling after each drop. The diluted reagent should be boiled before the urine is added in order to test its condition. If it remains clear, it is fit for use.

Criticism of Authorities.—In regard to the proportion of urine to be used in the test the authorities differ. Emerson specifies that the amount of urine must not exceed half the total volume of the undiluted mixture. Hawk and Saxe do not mention the amount to be added: W. Simon (9th edition) allows an equal volume of urine in all to be added if only small quantities of sugar are present.

Williamson insists that the quantity of urine should never be in excess of the Fehling's solution. Ogden mixes a finger-breadth of each solution in a test tube, boils and if the solution remains clear adds 20 to 30 drops of albumin-free urine without further boiling. Much sugar causes an immediate reduction, less than 1 per cent. requires 5 to 30 minutes, traces 18 to 24 hours. Note well that Ogden's formula for Fehling's solution is only half the strength of most of the formulas. C. E. Simon advises diluting the mixture with 4 times its volume of water, using a few c.c. boiled with a "small amount of urine." Most of the authorities consulted except Emerson and W. Simon advise dilution of the urine instead of the mixture, Simon not mentioning dilution at all.

Laboratory Note.—One thing the authorities appear to have overlooked and that is if the solution be diluted with 4 or 5 parts water, the opacity of the reaction is greatly affected. The author finds the following to be true:—

When undiluted Fehling's solution is boiled with half its volume of urine rich in glucose the entire fluid becomes opaque from

formation of a copious yellow or red precipitate, which after a time settles leaving a colorless or but faintly blue solution above it. But when Fehling's solution diluted with five times its volume of water is boiled and, as sometimes recommended, added, after cooling a little, to its own volume of boiled urine which also has been allowed to cool somewhat, little or no opacity is noticed; little when the urine is rich in sugar, none at all when the amount of sugar is small. A marked yellow *color* with a more or less copious white precipitate of phosphates occurs. As to the statement that by avoiding boiling we avoid confusion from presence of glycuronates, etc., this is not true altogether: since urine containing salicylic acid or its derivatives will turn golden brown when tested as above. Normal urine gives a faint green which soon fades.

The advantages of the test are its delicacy and, if performed according to Ogden, its reliability. Its great value is in a negative direction, absence of reaction indicating that, clinically speaking, sugar is absent. With dilution the danger of solution of the precipitate in a urine rich in uric acid or creatinine is avoided.

The disadvantages are the same as Trommer's and, as there is always more or less reduction from normal undiluted urine, dilution of either mixture or urine is imperative. An undiluted urine may also hold in solution the precipitate due to a trace of glucose because of the peculiar property of uric acid and creatinine above described. Prolonged boiling may cause a precipitate due to reducing agents other than glucose, as in the case of Trommer's test. (See above.) Albumin must be removed in the case of a negative result. The greenish flocculent precipitate of phosphates is the cause of much anxiety on part of the inexperienced. Unclean test-tubes cause much trouble with this test. The same drugs, glycuronic acid compounds, etc., as in Trommer's test are likely to produce a precipitate.

Changes in color alone are not referable to the presence of sugar but to other substances. The test must be made by daylight in order to observe a slight reduction by traces of glucose.

Some life insurance companies advise their examiners to neutralize an ammoniacal urine with acetic acid before testing.

This must be done carefully, as acetic acid affects the copper tests. No more should be added than enough to render the urine just neutral in reaction.

To summarize: if the test is performed without dilution a trace of glucose may escape detection, and, if the liquid is diluted before applying the test, time is lost and various colors instead of precipitates may confuse the operator.

If equal parts of almost any normal urine are added to the undiluted reagent with boiling, the whole turns green. Performance of the test by diluting and letting cool slightly, after boiling both reagent and urine, does not prevent changes in color due to glycuronates, etc., which can be proved by testing the urine of patients who are taking oil of gaultheria or salicylates in large amount, as in rheumatism.

The fallacy due to uric acid and creatinine holding cuprous oxide in solution, thus preventing a precipitate when glucose is present in small amount and destroyed by boiling in a strong alkaline solution, may be prevented by warming the urine instead of boiling. Hence, when the test is negative if performed in the usual way, it is well to repeat the process, first warming without actually boiling and second, if this be negative, trying both boiling and warming the diluted urine.

☞ **4. Haines' Test.**—This test possesses an advantage over Fehling's in that only one solution is used with only one way of making it, and the solution is practically permanent, a deposit of crystals taking place in it after a time, which, if anything, renders the test solution more delicate, decantation being all that is necessary to remove the solution from the deposit. Dr. Haines has tested samples of his solution more than ten years old and found their delicacy unimpaired. Use of glycerine appears to remedy the fault found by Benedict with Fehling's and Trommer's test.

☞ Haines' solution is made as follows: cupric sulphate, the purest obtainable, in amount 2 grammes, is dissolved in 15 c.c. of water and, after perfect solution, is well mixed with 15 c.c. of glycerine. To this mixture is then added 150 c.c. of liquor potassæ, a 5 per cent. solution of potassium hydroxide having a

specific gravity of 1036. Since one pound of Merck's c. p. glycerine measures about 360 c.c., it is convenient to dissolve 48 grammes of the copper salt in 360 c.c. of water, add one pound of the glycerine, and 3600 c.c. of liquor potassæ when a large supply of the reagent is needed, as in the case of college laboratories. The liquor potassæ may be made in quantity by dissolving one pound avoirdupois, 454 grammes, in 8625 c.c. of water, but as this quantity may be too large in some cases, count the number of sticks in a pound bottle of potassium hydroxide and dissolve half of them in 4313 c.c., or a quarter of them in 2156 c.c., etc., weighing being unnecessary. Haines' solution when made is of clear blue color containing the cupric hydroxide in solution in glycerine and water.

In American measures the solution is made as follows: perfect solution of 30 grains of the copper salt is made in one-half fluidounce of distilled water; to this is added one-half fluidounce of pure glycerine; the two are mixed thoroughly and added to five fluidounces of the liquor potassæ. Let stand and if a reddish deposit is seen in it after a time, decant off the supernatant liquid. If it appears again, decant again or filter through cotton.

☞ **Technique of the Haines' Test.**—Measure out 3.75 cc. (1 fl. drachm) of the solution into a test-tube, boil it and, if it remains clear, proceed with the test; otherwise it is unfit for use, or else the test-tube is unclean, which is the more likely unless the solution has been tampered with by ignorant or designing persons.

Haines' solution is permanent for years, and the reddish precipitate occurring on standing is of no consequence chemically, since the clear liquid can be decanted off from it. If Merck's reagent (cupric sulphate) is used, the precipitate may be absent altogether, or very slight. Filter through absorbent cotton if necessary.

Having ascertained by boiling that the test-liquid is of good quality, while still hot add one drop of the suspected urine to it. If much sugar is present in the urine, a change at once takes place; the whole liquid becomes turbid and changes color to a yellow, reddish-yellow, or brown-yellow. If no such

change takes place after adding a drop of urine, add another drop and bring to boil again, and so on until the turbidity and discoloration are seen; urine which contains but a moderate quantity of sugar may require four drops to be added, boiling after each drop. Or it may be necessary in case but a small quantity of sugar be present to add eight drops of urine, boiling after each drop, and after eight drops are added to boil for thirty seconds before any change be seen. If, however, no change is seen even then, let the tube cool, when, if but a minute quantity of sugar is present the liquid becomes greenish and turbid. But if no sugar is present the brilliant blue transparency is unaffected on cooling, a fading of the color being, however, noticed in some cases of concentrated urines. This fading is not due to sugar, but to uric acid, etc.

☞ **Precautions in using Haines' test:**

1. The test depends on the reduction of the cupric sulphate to cuprous oxide. Normal urine has a slight reducing power on the solution in case of prolonged boiling, therefore, do not boil too long, thirty seconds being enough.

2. Do not forget to set the tube aside after the test has been made and let it cool. A small quantity of sugar causes a turbidity only on cooling. A dirty test-tube is more often responsible for the changes on cooling.

3. Do not use a sample of the liquid which contains a reddish sediment, lest the latter appear in the test-tube and be mistaken for a reduction. Filter through absorbent cotton before using.

4. Do not mistake the whitish flocks of phosphates precipitated in all urine by this test for sugar.

5. Do not use a dirty test-tube, since Haines' liquid is exceedingly sensitive to the presence of numerous organic substances. The test-tube must be *thoroughly* cleaned beforehand, preferably with hot water.

6. Do not use more than 8 or 10 drops of urine; a larger quantity of even normal urine may cause reduction.

7. Do not add any chemicals whatever before or after the test.

8. Use a clamp for holding the test-tube and use not too great a heat suddenly. An alcohol lamp is better than a Bunsen burner, unless the latter be turned low.

9. Point the solution away from any person near, since it sometimes "bumps," *i. e.*, leaps out of the tube with explosive violence if heated too suddenly.

10. In doubtful cases, *i. e.*, when a precipitate occurs with from 8 to 10 drops or only upon cooling, try the test again, either adding only 6 drops of the urine or diluting the reagent with 3 parts water, mixing well, boiling the upper portion and adding the urine, drop by drop, up to 6 or 8 drops.

☞ **Advantages.**—As a rule, if glucose is present in quantity, 6 drops of the suspected urine will show it. So little urine is added in proportion to the reagent that uric acid, creatinine, and other reducing bodies are not likely to be present in sufficient quantity to interfere with the test either positively or negatively (by dissolving the precipitate).

The same may be said of ammonia in the urine. But this applies to cases in which 6 drops only of urine are added to 3.75 c.c. of reagent (1 fluidrachm). A great advantage is time saved in not being obliged to dilute either the solution or the urine, except possibly in a few doubtful cases. The phosphatic precipitate attracts less attention than in Fehling's test, since so little urine is used.

☞ **Disadvantages.**—The great disadvantage in using Haines' solution with a class of students is the trouble in adjusting the proportions, since few students will take the precaution to measure both reagent and urine. Again, the solution reacts readily with a number of organic substances used in laboratories and unless the test-tubes are washed out with *hot water* strange reactions may occur. The deposit of reddish crystals occurring in the supply bottle should be removed from the liquid, for when allowed to remain they cause trouble as above described in Precaution 3. Glycuronates are likely to cause a reaction, as well as other sugars, especially **lactose**, and these are the most serious sources of error. Many normal urines change the color from a deep brilliant blue to a dull light blue, but this does not

imply the presence of a trace of sugar. When the reaction is due to glycuronates, drugs, etc., it is likely to occur only after addition of 8 or 10 drops of urine and sometimes not until the urine has cooled, but lactose may cause a copious precipitate with fewer drops.

Summary.—The author has used Haines' test for 32 years and finds it on the whole the most satisfactory of any one test. Doubtful cases may be rendered more certain (a) by discontinuing the use of drugs or beer during the collection of urine for the test and (b) by administering 100 grammes of glucose according to the method of Naunyn. Lactose may be suspected by the fact of lactation in the patient and by absence of diabetic symptoms. Fermentation is necessary for deciding that the reaction is due to lactose. The author obtained a positive reaction with Haines' test in a case of alkaptonuria, but was not deceived by it, since the urine in no way suggested the presence of sugar, hence confirmatory evidence was sought and not found. A great advantage consists in the absence of the many colors, which confuse the operator using Fehling's test, one way and another. Haines' test either produces an opacity or it does not, and when the opacity is absent no change in color occurs except the dimming of the blue. **Opacity only is what constitutes reaction with Haines'**; if the fluid is clear, barring a faint cloudiness due to phosphates, there is no sugar. On the other hand, when Fehling's test is used, diluted strongly as recommended, it virtually becomes a color test even when plenty of sugar is present: this may not affect the experienced operator, but the change is not so striking as when an opacity due to a precipitate is obtained, hence not so likely to be interpreted correctly by the inexperienced.

☞ The most serious objection to Haines' test is the fact that the solution must be "made right" and "used right." Careless or dishonest druggists may use impure copper sulphate, weak glycerine and cheap alkali, with production of a liquid which gives strange and confusing results when boiled with urine. Again, inexperienced persons usually insist upon using altogether too much urine in making the test. Such conditions and the fact that the test is an American one, not made in Germany,

have caused much distrust on part of the unscientific. Order the test-liquid of E. H. Sargent & Co., 125 West Lake street, Chicago, and specify that Merck's reagent chemicals are to be used.

5. Benedict's Test.—On the theory that it is not glucose itself which reduces the copper salt, but a reducing substance formed from the action of alkali upon the glucose and which is unstable in the presence of strong alkali, Benedict has devised a test in which weaker alkali than the hydroxides is used.

His formula is as follows: dissolve 8.65 grammes of pure cupric sulphate in 50 c.c. of distilled water, filter, and make up to 75 c.c. with distilled water. Dissolve also 86.50 grammes of pure sodium citrate and 50.00 grammes of pure **anhydrous** sodium carbonate in 300 c.c. of water by the aid of heat, filter, and make up with distilled water to 425 c.c. The copper solution is then poured slowly with constant stirring into the soda salts solution.

To perform the test proceed as follows: pour five c.c. of the solution into a test tube, add from 8 to 10 drops, but no more, of the urine and boil vigorously for two minutes. Let cool spontaneously. If sugar is present, the entire body of the solution will be filled with a precipitate which may be red, yellow, or greenish. If sugar is small in amount, less than 0.5 per cent., the color of the precipitate is greenish. If the amount is less than 0.3 per cent. the precipitate forms only on cooling. If no sugar is present, the solution is either perfectly clear or else shows a faint blue turbidity due to urates. The reagent is much more sensitive than either Fehling's or Haines'. The test liquid is permanent. Normal urines are not reduced by the test. Homogentisic acid (alkapton), lactose and glycuronates interfere. But the test is not affected by uric acid nor by creatinine. Benedict claims also that glycuronates must be in greatly increased quantity to affect his test and insists that chloroform, choral, aldehyd, and formaldehyd are without appreciable effect.

The author has used Benedict's test in a few cases and finds it certainly much more delicate than Haines' or Fehling's In several instances reactions obtained have been obviously due to traces of saccharine substances present in bottles or other containers of the urine

6. Nylander's Test.—This is a **bismuth** test and is valuable both as a negative one and in confirmation of the copper tests. The test solution is made by dissolving 4 grammes of Rochelle salt in 100 c.c. of a warm 10 per cent. solution of sodium or potassium hydroxide and saturating with bismuth subnitrate, of which 2 grammes are necessary. When cooled, the mixture is filtered and kept in an amber bottle. It is permanent even for years. To 10 c.c. of the urine in a test-tube add 1 c.c. of the reagent and place for five minutes in the boiling water-bath.

The measurement of the reagent must be accurate in testing for traces of glucose. If sugar is present, the mixture will turn black at once from formation of a black precipitate of metallic bismuth. The test is said to detect from 0.08 to 0.025 per cent. of sugar, traces being indicated by a slight gray color on the upper surface of precipitated phosphates. The principle of the test is the formation of bismuthous hydroxide, $Bi(OH)_3$, and the reduction of it to metallic bismuth. The advantages of the test are that it is not positive with uric acid, creatinine, pyrocatechine, hydroquinone, and alkapton. Moreover, it is a specially valuable negative test.

The *disadvantages* of the test are many: ammoniacal urines can not be tested by this method, nor even urines containing a large amount of ammonia, as in diabetic acidosis, hence this test must be not depended upon to the exclusion of others. Urines containing hydrogen sulphide or albumin cause a positive reaction. Concentrated normal urine gives a doubtful or even positive reaction and must be diluted. Nearly all the urinary pigments (uroerythrin, urobilin, blood-pigment, melanin, hematoporphyrin, indican) may yield a similar reaction. Commonly eaten vegetables, as asparagus and rhubarb, and frequently taken drugs, as senna, sulphonal and quinine in quantity, give the reaction, as do also most all the drugs which interfere with the copper tests. Other sugars than glucose also reduce the solution. Chloroform added as a preservative prevents the reaction, but may be boiled out of the urine before the test is made, five minutes boiling being usually enough. Turpentine inhaled causes a positive reaction according to Kamberg.

7. The Boettger-Bruecke Tests.—Boettger's test was merely to boil equal volumes of urine and liquor potassæ and to add a pinch of bismuth subnitrate while boiling. In the presence of sugar a black or gray precipitate appears. As sulphur even in traces yields the same reaction Bruecke uses Frohn's reagent, which consists of 1.5 grammes of freshly precipitated bismuth subnitrate mixed with 20 c.c. of water and heated to boiling, after which 7 grammes of potassium iodide and 20 drops of strong hydrochloric acid are added. Ten c.c. of water are poured into one test-tube and the same amount of the urine into another. To the water is added a drop of Frohn's reagent which will cause a precipitate which is to be redissolved by addition of hydrochloric acid, drop by drop. Add the same quantity of hydrochloric acid to the urine, then add the bismuth solution until precipitation is complete, and filter. The filtrate should not be rendered turbid either by acid or reagent. Boil it with excess of alkali as in Boettger's test and, if sugar is present, a gray or black precipitate occurs.

This test is said to be delicate, detecting 0.4 per cent. of glucose in aqueous solution, but the performance takes time and requires skill.

8. Miscellaneous Tests.—A large number of tests for sugar have been suggested. The *permanganate* test consists in adding one drop of 1 per cent. permanganate solution to 5 c.c. of very dilute sodium hydroxide and further adding the urine, drop by drop. In the presence of sugar a black color without a precipitate occurs, while other reducing bodies cause a precipitate. Penzoldt suggested a test with *diazo-benzol sulphonic acid.* Johnson with *picric acid,* Molisch with *alphanaphthol* or *thymol* (purple or violet), and Muelder with *indigo-carmine.* These are deemed inferior to the tests above described and are but little used. *Picric acid* may be used as follows: the urine is mixed with a few drops of a saturated aqueous solution of picric acid, a little liquor potassæ is added and the whole gently heated. A marked reddish or reddish-brown color (picramic acid) denotes presence of sugar, but a red color appearing in the cold and disappearing in 20 minutes is due to creatinine. If sugar be

present, this red color due to creatinine turns still more red when heated. *Orthonitrophenylpropionic acid* is used and has been recommended by Williamson as a confirmatory test when sugar is present in amount 0.5 per cent. or over. A one-half per cent. solution in sodium hydroxide, 10 per cent., is used and the test made as with Haines' test liquid. A dark blue color indicates sugar. *Methylene blue* in diluted alkaline solution is decolorized by glucose in diluted urines on boiling. *Saffranin* dissolved in water gives a blood-red which is decolorized in alkaline solution when heated in the presence of glucose.

9. Detection of Sugar in Normal Urine.—There are two methods used by physiologists, viz.: Worm-Mueller's and that of Patein-Dufau. The former shows a minimum of 0.025 per cent. The latter is based upon the following principles: precipitate the proteins with nitrate of mercury, neutralize with sodium hydroxide, remove excess of mercury and then determine the sugar. This method gives results as low as 0.01 per cent. (See article by B. Schoendorff in *Archiv. für die gesammte Physiologie,* CXXI, p. 572.)

MICRO-CHEMICAL TESTS.

The phenylhydrazine hydrochlorate tests are as follows:

1. Neumann-Thierfelder Method.—To 5 c.c. of urine add 2 c.c. of 50 per cent. acetic acid saturated with sodium acetate and 2 drops of pure phenylhydrazine (Merck's reagent) which occurs in the form of a colorless or slightly yellowish highly refracting liquid. Concentrate to 3 c.c. by boiling and then cool rapidly. In the presence of from 0.02 to 0.05 per cent. of glucose the crystals may be seen in from 5 to 10 minutes. For the rapid performance of this test a specially graduated test-tube may be used. The crystals should be filtered out, dissolved in hot 60 per cent. alcohol, allowed to recrystallize by adding water, after boiling away the alcohol. The pure crystals melt at from 204° to 205° C. (399.2° to 401° F.); if impure they melt at from 173° to 194° C. (341.4° to 381° F.). The crystals are yellow needles which, under the microscope, appear arranged as sunbursts, fans, sheaves, etc., (Fig. 26), but the crystals are not

characteristic of glucose alone, other sugars yielding similar crystals, hence the determination of the melting point becomes important. In order to do this an apparatus has been described by Emerson as follows:

Into a small flask three-quarters full of concentrated sulphuric acid, a test-tube, half-full of the same acid, is fitted by means of a perforated stopper. The whole is held by a clamp to an upright support. Into the test-tube a thermometer (Centigrade) is carefully dipped, submerging the mercury bulb in the

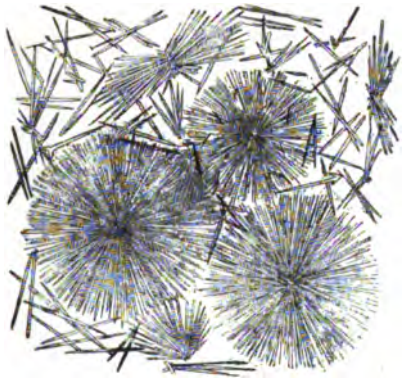


FIG. 26.—Phenylglucosazone Crystals (v. Jaksch).

acid. To the lower part of the thermometer and parallel to it is fastened a pointed glass tube of small caliber, closed at the tip, containing the crystals. This tube is attached to the thermometer by means of a rubber band (above the acid). The flask is warmed slowly with a Bunsen burner and the point noted at which the crystals melt. A temperature of about 200°-205° C. is characteristic for glucose, levulose, or lactose, while pentoses give lower melting points (159° to 160° C.). Since

other sugars can be distinguished by various tests the phenylhydrazine test becomes important for differentiation of *pentoses* from glucose.

2. Another method advised by C. E. Simon is to mix 5 drops of pure phenylhydrazine in a test-tube with 10 drops of glacial acetic acid and 1 c.c. of a saturated solution of common salt. To this are added 3 c.c. of the urine, the mixture boiled 2 minutes and let cool. In the presence of more than 0.5 per cent. of glucose the crystals begin to separate out in one or two minutes. If smaller amounts are present, it is necessary to wait longer.

The author prefers the liquid tests in which the pure phenylhydrazine is used, since the handling of the various crystals is less convenient. Williamson's test with the crystals is criticised severely by Ogden.

The crystals of the osazone may be few and small, requiring a high power (450 diameters) for indentification in some cases, but on long standing, *e. g.*, over night, larger crystals may be formed, plainly visible with 150 diameters. The advantages of the phenylhydrazine tests are delicacy and absence of reaction with uric acid, creatinine, hippuric acid, pyrocatechine, and alkapton.

3. Williamson's Test.—In the absence of the liquid phenylhydrazine the method of Williamson with crystals may be used as follows: a test-tube of ordinary size is filled for about half an inch with crystals of phenylhydrazine hydrochlorate and an equal volume of sodium acetate, also in crystals, added. The tube then half filled with urine, boiled for two minutes, and set aside for several hours, at the end of which time the needle-shaped crystals of sulphur-yellow color arranged for the most part in sheaves may be seen with the microscope.

The author with this test obtained plenty of crystals in a sample of urine which showed only one-eighth of 1 per cent. of sugar with the Einhorn saccharimeter and doubtful reactions with the copper tests. But no crystals could be identified with a low power (150 diameters) until the tube had stood over night.

☞ **Fermentation Tests.**—The readiest method of applying the fermentation test is by the use of a saccharimeter, of which

there are several on the market. The ones most readily obtainable in Chicago and vicinity are *Einhorn's* and *Nelson Baker & Co.'s*.

The writer uses the former (Fig. 27) for *qualitative* testing in doubtful cases, as, for example, when the reaction with Haines' test is obtained with from 6 to 10 drops of urine or after cooling only. To 10 c.c. of the well boiled and cooled urine add 1 gramme of fresh yeast, mix well without shaking and pour into the cup of the saccharimeter; slant the instrument until the liquid runs into the tube, taking care that no bubbles of air collect at the top. A companion tube with normal urine also boiled and cooled and with the same yeast is arranged in the same way. Let the tubes stand at room temperature, the best range being from 15° to 34° C. (59° to 93° F.).

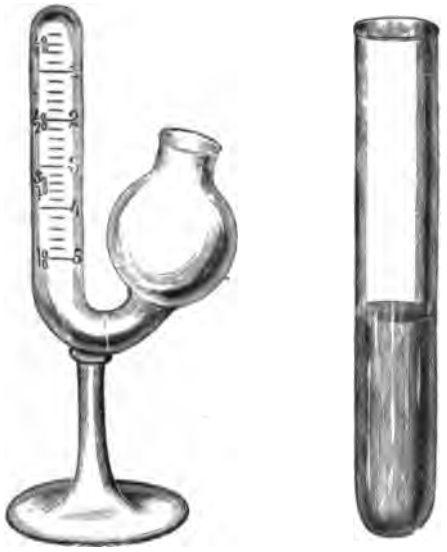


FIG. 27.—Einhorn's Saccharimeter. Test-Tube, holding 10 c.c. to the mark.

If sugar is present, bubbles of carbon dioxide form at the top of the tube in the course of about six hours or less. There should be no bubbles, or at most a small bubble in the tube containing normal urine. If the urine is feebly acid or alkaline, acidulate it beforehand with a little tartaric acid.

If the bubbles form only after a protracted period, as *e. g.*, a day or two, the result may be due to gas-forming bacteria, hence for positive results determine the presence of gas within a few hours. The principle of the test is the splitting of the glucose into alcohol and carbonic acid according to the equation $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$.

The advantages of the test are that when positive it distinguishes glucose from lactose, glycuronates, pentoses and homogentisic acid. The presence of carbon dioxide in the tube can be proved by adding a small fragment of caustic soda, when the gas will be absorbed and the liquid rise in the tube again.

The disadvantages are the lack of delicacy, the amount of time required and the possibilities of inefficiency on the part of the yeast. If the last is suspected, try a third experiment, adding a little glucose to boiled and cooled normal urine and test it with the same yeast.

To remedy the disadvantage of absorption of carbonic acid in small amount from a trace of glucose the combined method with Nylander's test is used.

Fermentation and Nylander's Test Combined.—If Nylander's test is positive before the urine is fermented, but negative after 12 to 24 hours of fermentation, sugar was present in traces even if no bubbles appear to be formed. If, on the other hand, Nylander's test is positive both before and after fermentation and no bubbles are seen, some non-fermentable reducing substance is present, as, *e. g.*, lactose.

Polariscopic Test.—The principle of this test is the rotation of polarized light by glucose to the right. The so-called half-shadow saccharimeter is used in modern work. Normal urine is slightly lævorotatory (0.005° to 0.18°). Albumin is lævorotatory and must be removed by boiling the slightly acidulated urine and filtering after cooling.

The Bausch and Lomb apparatus is a half-shadow instrument with wedge compensation, which can be used with any kind of light. (See "Quantitative Determination of Sugar.")

CHAPTER XXII.

THE QUANTITATIVE DETERMINATION OF SUGAR.

Unsatisfactory nature of methods employed for determination of the quantity of sugar in the urine.
Volumetric analysis:—use of Fehling's solution (formula of Ogden); calculation of results.
Methods for determining with exactness the end-reaction in Fehling's method.
Beck's modification of Fehling's method.
Purdy's method with ammonia. Benedict's method.
The Purdy-Haines method with ammonia; calculation of results.
Polariscopic determination; various instruments and technique.
Fermentation methods; Roberts' differential density; various instruments used for quantitative fermentation processes.

As in the case of albumin, quantitative analysis for sugar in urine is unsatisfactory from a purely chemical standpoint, and the best evidence of it is the number of methods which have been proposed. The author does not necessarily endorse the various methods about to be described. It does not follow that because a method gives exact results with a solution of glucose in water that it will also yield exact figures in as complex a fluid as the urine.

Other substances besides sugar affect the end-reactions in the volumetric tests, so that the general practitioner is advised to adhere to the fermentation processes. When the patient, however, demands an analysis "while he waits" the volumetric methods must be employed.

The methods in vogue are: (1) volumetric by the copper tests described above, (2) fermentation, (3) polariscopic, and (4) mathematical by rough estimate from the specific gravity.

Volumetric Analysis.—1. The standard method involves the use of *Fehling's solution* and is difficult on account of the trouble in determining the end-reaction and requires half an hour's time. The modified Fehling's solution is used, *i. e.*, the formula of Og-

den referred to in which the copper and the alkaline solutions each measure 1000 c.c. If the urine have a specific gravity of more than 1030, dilute it with 9 times its volume of distilled water, otherwise with four times only, mixing well after dilution. Pour the diluted urine into a Schellbach burette with a glass stop-cock, filling it to the zero mark and see that all air is expelled from below the cock. Measure out, preferably with a pipette, exactly 10 c.c. of each of the solutions (copper and alkali), and mix them well in a 250 c.c. flask. Dilute with water to a total volume of 80 c.c. (It is well to have four of these flasks thus arranged on a wire gauze over the flame kept as near the boiling point as possible.) Place one of the flasks under the burette containing the urine and bring it to a boil. After a minute or two if there is no change in color or appearance, run in the diluted urine, drop by drop, until the meniscus loses its blue color, *i. e.*, the uppermost layer of fluid from the precipitate first settles. This is best seen by placing the flask in front of a window between which and the flask is held a piece of white paper with the eye on a level with the meniscus. If the eye is in the right position, there is soon seen a line of clear fiuid above the precipitate which rapidly settles and the color of this line is easily determined. At first it is blue, but slowly grows lighter in color until finally it is colorless when the reaction is over. The flask must be removed from the flame in order to see this line. The four flasks mentioned above may be used for greater accuracy. Thus, if the reaction be over when somewhere between 5.8 and 6.3 c.c. of urine have been added, raise the contents of all the flasks to boiling and add 5.9 cc., 6, 6.1, 6.2 cc. of the diluted urine to them, respectively. All four can settle at once; considerable time is thus saved. The menicus must be colorless; if yellow, it means that too much urine has been added.

Albumin, if in amount more than a trace, should be removed by boiling the urine, made faintly acid, if necessary, with acetic acid until a flocculent coagulum is obtained by adding the acid, drop by drop, until such a coagulum occurs. Filter and use the filtrate for the quantitative determination of sugar.

Calculation of results: since it takes 50 milligrammes, 0.050

gramme of glucose, to reduce 20 c.c. of the modified Fehling's solution *(formula of Ogden)*, and if it required 17 c.c. of the diluted urine to decolorize the meniscus, then the total amount of undiluted urine required is 1.7 c.c. That is, the 1.7 c.c. must contain 50 milligrammes of sugar: that is, 1.7 : 0.05 :: 100 : x or x = 5 divided by 1.7 = 2.92 per cent.

Hence, a ready method of calculation of percentage in all cases is to divide the figure 5 by the number of c.c. of undiluted urine required.

To obtain the quantity of sugar in 24 hours multiply the per cent. by the weight of the 24 hours' urine in grammes and divide by 100. If the patient passes 2500 c.c. of a specific gravity of 1030, then 2500 times 1.030 or 2575 grammes is the weight of this urine, which multiplied by 2.92 and divided by 100 equals 75.19 grammes per 24 hours. Approximately and with sufficient correctness for clinical purposes the number of cc. of urine in 24 hours, multiplied by ten times the percentage of sugar, gives the sugar per liter, from which the total for 24 hours may be calculated in the usual way.

Thus the 2.92 (above) times 10 = 29.20 grammes of sugar per liter and since 2500 c.c. = 2.5 liters then 29.20 times 2.5 = 73.

Laboratory Note.—Other methods for determining with exactness the end-reaction are as follows: (1) the ferrocyanide method by which the mixture in small amount is filtered through two thicknesses of filter paper, the filtrate acidulated with a drop or two of acetic acid and tested for copper with ferrocyanide; a brown color showing unreduced copper, hence insufficient addition of urine; (2) the iodine method by which a drop or two of the mixture is added to 1 c.c. of a starch-iodide solution acidified with 2 to 3 drops of acetic acid; when a purple or blue color appears it shows liberated iodine, which, in turn, means unreduced copper, *i. e.*, too little urine added. The iodide of starch is made by adding to 100 c.c. of starch paste in the cold 25 grammes of potassium iodide and diluting to 250 c.c. The starch paste should contain 0.1 gramme of starch rubbed up in the usual way with a little cold water with continual stirring.

2. **Beck's Modification of Fehling's Method.**—This requires

four centrifugal tubes graduated at 2 c.c., a pipette of 2 c.c. capacity graduated into twentieths of a c.c., and a wire tube-holder to support the tubes when placed in a beaker. Fill the four tubes to the mark 2 c.c. with the standard Fehling's solution, place in the tube-holder, and suspend in a beaker one-third full of boiling water. Number the tubes 1, 2, 3 and 4, and add $^4/_{20}$ of a c.c. of urine to the first, $^5/_{20}$ to the second, $^6/_{20}$ to the third, and $^7/_{20}$ to the fourth. Shake well and suspend in the boiling water for at least three minutes; then remove and centrifugalize until the precipitate is sedimented. If the supernatant liquid is still blue in all tubes add four more twentieths to each tube and centrifugalize again. Continue the process until some one tube is completely decolorized and figure the amount of sugar from this one: 1000 c.c. of *Standard Fehling's solution* (not Ogden's formula) contain 34.67 grammes of cupric sulphate ($CuSO_4 \cdot 5H_2O$), then 1 c.c. of solution is reduced by 0.005 of glucose or 2 c.c. by 0.01 gramme. Hence the number of twentieths of a c.c. used must contain 0.01 gramme of glucose. Hence the percentage of sugar is readily determined by dividing twenty by the number of twentieths used; if 8 twentieths were necessary, the percentage is, therefore, 2.5. Since 4 twentieths would indicate 5 per cent. only, the urine should be diluted with equal parts of water when of high specific gravity and results obtained multiplied by 2. Thus, if 4 twentieths of such diluted urine were used the true per cent. obtained would be 5 times 2, or 10.

Boston has a clinical quantitative method to be found in his book on *Clinical Diagnosis*, and Benedict has devised a method which can be read in Hawk's *Physiological Chemistry*. Neither of them is especially advantageous, *i. e.*, in the sense of surpassing the Haines'-Purdy method described below.

Benedict's method is somewhat laborious and is claimed by Webster to be no more accurate than the Purdy-Haines' method which is much simpler. See, however, below.

3. **Purdy's Method.**—Purdy advised the use of a liquid consisting of cupric sulphate 4.752 grammes; potassium hydroxide 23.5 grammes; ammonia (of specific gravity 0.090) 350 c.c.; glycerine 38.00 c.c.; distilled water to make a liter. Place 35 c.c. in

a 200 c.c. flask and add about twice its volume of distilled water (Fig. 28). Into the neck of the flask insert a doubly perforated cork and through one perforation introduce a tube bent at right angles to carry off the steam and fumes of ammonia, and into the second perforation introduce the tip of the burette holding the urine, which should be diluted with 2 or 3 volumes of water if a large amount of sugar is present. Heat the solution in the flask and add the urine, drop by drop, until the blue color begins to fade, then more slowly until all color is lost. Thirty-five c.c. of the solution is reduced by 0.02 gm. dextrose, hence, if 10 c.c. of urine were used $10 : 0.02 = 100 : x$, then 0.2 per cent. of sugar was present.

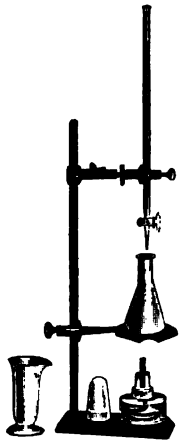


FIG. 28.—Purdy's Quantitative Method.

☞ 4. **Purdy-Haines' Method.**—When the patient is in a hurry to know the "exact per cent." of sugar in his urine the quickest method is by use of Haines' modification of Purdy's. Dissolve 8.314 grammes of cupric sulphate, Merck's reagent, 25 grammes pure potassium hydroxide, 40 of pure glycerine, and 350 of strong ammonia in water to make a liter. Take 10 c.c. of this liquid and add 50 c.c. of water to it. The rest of the process is the same as in the case of Purdy's. It is well to repeat the process adding the second time 1 c.c. less of the urine and

then adding the last portions, two drops at a time, allowing from 3 to 5 seconds to elapse between each addition.

A feature of Haines' method is that his solution corresponds to 0.01 gramme of glucose: hence, if 10 c.c. of urine were used 10 : 0.01 = 100 : x or x = 0.1 per cent. Hence a table is readily constructed:

0.1 c.c. of urine = 10 per cent. sugar; 0.2 c.c. = 5 per cent.; 0.3 c.c. = 3.33 per cent.; 0.4 c.c. = 2.5 per cent.; 0.5 c.c. = 2 per cent.; 0.6 c.c. = 1.66 per cent.; 0.7 c.c. = 1.43 per cent.; 0.8 c.c. = 1.25 per cent.; 0.9 c.c. = 1.11 per cent.; 1 c.c. = one per cent.

Reckoning two drops of urine as 0.1 c.c., a medicine dropper may be used for delivering the urine. It is better, however, to dilute the urine abundantly with three times or more its volume of water and deliver from a Schellbach burette graduated in tenths, multiplying results by 4 or more according to the volumes used. Then if 0.7 c.c. of a urine diluted with three times its volume of water decolorized the reagent, then 1.43 times 4 = 5.72 per cent. of sugar present. Close reading is a necessity, hence a burette of small diameter with wide intervals between the graduations is desirable, or else the urine must be greatly diluted.

Laboratory Note.—The Purdy methods are subject to error from presence of glycuronates in the urine, hence care should be taken not to add chloral, camphor, chloroform or other reducing agents as preservatives, nor to allow the patient to take them internally when the urine is to be tested quantitatively.

The method of Purdy modified by Haines is advised for every-day hurried clinical use. Fermentation (Robert's method) is less subject to error from glycuronates, etc., and is perhaps on the whole, therefore, more trustworthy, but is much slower.

5. Benedict's Latest Method.—In the *Journal of the American Medical Association* for October 11th, 1911, we find still another method by Benedict:

The solution for quantitative work has the following composition:

	GM. OR C.C.
Copper sulphate (pure crystallized)	18.0
Sodium carbonate (crystallized)*	200.0
Sodium or potassium citrate	200.0
Potassium sulphocyanate	125.0
Five per cent. potassium ferrocyanide solution	5.0
Distilled water to make a total volume of	1,000.0

With the aid of heat dissolved the carbonate, citrate and sulphocyanate in enough water to make about 800 c.c. of the mixture, and filter if necessary. Dissolve the copper sulphate separately in about 100 c.c. of water and pour the solution slowly into the other liquid, with constant stirring. Add the ferrocyanide solution, cool and dilute to exactly 1 liter. Of the various constituents, the copper salt only need be weighed with exactness. Twenty-five c.c. of the reagent are reduced by 50 mg. of glucose.

Sugar estimations are conducted as follows: The urine, 10 c.c. of which should be diluted with water to 100 c.c. (unless the sugar content is believed to be low), is poured into a 50 c.c. burette up to the zero mark. Twenty-five c.c. of the reagent are measured with a pipette into a porcelain evaporation dish (25-30 cm. in diameter), 10 to 20 gm. of crystallized sodium carbonate (or one-half the weight of the anhydrous salt) are added together with a small quantity of powdered pumice-stone or talcum, and the mixture heated to boiling over a free flame until the carbonate has entirely dissolved. The diluted urine is now run in from the burette, rather rapidly until a chalk-white precipitate forms, and the blue color of the mixture begins to lessen perceptibly, after which the solution from the burette must be run in a few drops at a time, until the disappearance of the last trace of blue color, which marks the end-point. The solution must be kept vigorously boiling throughout the entire titration. If the mixture becomes too concentrated during the process, water may be added from time to time to replace the volume lost by evaporation. The calculation of the percentage of sugar in the original sample of urine is very simple. The 25 c.c. of copper solution are

*One-half the weight of the anhydrous salt may be used.

reduced by exactly 50 mg. of glucose. Therefore the volume run out of the burette to effect the reduction contained 50 mg. of the sugar. When the urine is diluted 1:10, as in the usual titration of diabetic urines, the formula for calculating the per cent. of sugar is the following:

$$\frac{0.050}{X} \times 1{,}000 = \text{per cent. in original sample.}$$

wherein X is the number of cubic centimeters of the diluted urine required to reduce 25 c.c. of the copper solution.

In the use of this method chloroform must not be present during the titration. If used as a preservative in the urine it may be removed by boiling a sample for a few minutes and then diluting to its original volume.

Like the reagent for qualitative employment the one for quantitative work will keep indefinitely after its preparation. As regards the accuracy of the method, it may be stated that repeated determinations, and comparisons with results by the polariscope and by Allihn's gravimetric process, have shown the method to be probably more exact than any other titration method available for sugar work.

6. **The Safranin Method.**—An alkaline solution of safranin standardized by means of a glucose solution of known concentration is heated and the urine run into it. The deep red color changes finally to yellowish red. Albumin need not be removed and neither uric acid nor creatinine reduces the safranin. (Hasselbach and Lindhart.)

Polariscopic Method.—The so-called half-shadow instrument consists of a Nichol's prism which acts as a polarizer, a tube to carry the urine, and a second Nichol's prism or analyzer by which the amount of rotation caused by the fluid is measured. The zero of the instrument is fixed at the point at which the two lateral halves of the field of the eyepiece are equally illuminated. The position of the zero on the scale must be determined before using the instrument, since this may be altered by moving the apparatus. The observation tube is then filled with urine which has been acidified with acetic acid and precipitated with a little

lead acetate in crystalline form and filtered. The tube, if not perfectly dry, should be washed out with distilled water and the latter rinsed out with the filtered urine at least once or twice. One end of the tube is closed with a cap. The other end is filled a little more than full and a glass cover slid on it sideways in order to avoid leaving a bubble of water in the tube. The brass cap is screwed on not too firmly. If available, caps which slide on are to be preferred. After the tube is placed in position the eyepiece is drawn out until the two divisions of the field are separated by a sharp line, rotating the handle through 20-30 degrees for this purpose so as to get a strong contrast between the two fields. The handle is then turned until the vernier approaches zero and the brightness of the two halves of the field rendered equal as nearly as possible. The vernier is then read by means of a hand lens and the observation noted. This should be repeated three or four times and an arithmetical mean taken of the results. The observation tube must be cleaned immediately after use and care should be taken that the caps are not left tightly screwed down.

Calculation of results: the specific rotation of glucose is [a] $= +$ 52.74, for practical purposes + 53.

The formula used is as follows: $g = \frac{a \text{ times } 100}{53 \text{ times } l}$

in which g = amount of glucose in grammes in 100 c.c. of urine, a = the angle of rotation, and l = the length of the tube used.

Two tubes are used, a longer and a shorter; the short one corresponds to 2 per cent. of glucose for each degree of rotation, the longer one to 1 per cent. (Webster). Lead acetate is necessary when the longer tube is used.

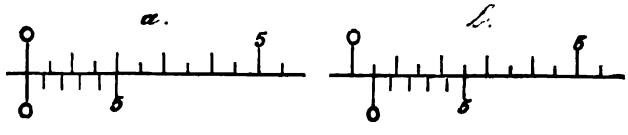
The Bausch & Lomb Instrument.—A polariscope is now constructed by Bausch & Lomb with quartz wedge compensation by means of which any ordinary light—gas, petroleum or electric—may be used. The polarizer used by the author consists of a Jelett-Cornu half-shadow prism. The quartz wedge may be moved horizontally by a rack and pinion.

Fastened to the movable wedge is a linear scale of ivory; this shows graduations in tenths by means of a vernier and a magni-

fying glass. The zero point of vernier can be set by means of a micrometer screw.

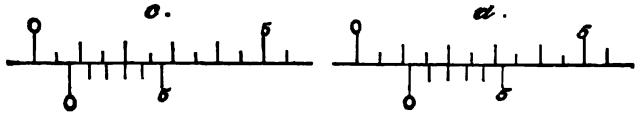
Two observation tubes are attached to the apparatus, namely, of 200 millimeters and 100 millimeters in length, respectively. By use of the 200 m.m. tube the per cent. of sugar in 100 c.c. of fluid examined is obtained.

It is claimed that by means of this apparatus a percentage of 0.05 is obtainable. In order to be able to read off conveniently with the magnifying glass, the scale is sub-divided into half graduations while the vernier to the left and to the right of the zero point contains each way five graduation points, which correspond each way to four graduations of the scale and by means of which the possibility of a direct reading off of tenth-graduations and estimations of twentieth-graduations is obtained.



For the determination of the two zero points both halves of the field of vision must be equally illuminated. If this is not the case, then the optical equality must be rearranged and the zero point corrected.

When the zero of the vernier falls upon the first half graduation of the scale, then 0.5 per cent. of dextrose is indicated.



When the zero of the vernier stretches beyond the first half graduation of the scale and the third vernier graduation coincides with the fourth scale graduation, then 0.5 per cent. + 0.3 per cent. = 0.8 per cent. dextrose.

When the zero point of the vernier has passed over the first whole graduation of the scale and the second vernier graduation coincides with the fourth scale graduation, then 1.2 per cent. of dextrose is present.

The source of light should be removed from 13 to 14 centimeters from the apparatus. By mechanical means a part of the source of light is reflected upon the scale. The same happens in the case of the other tubes.

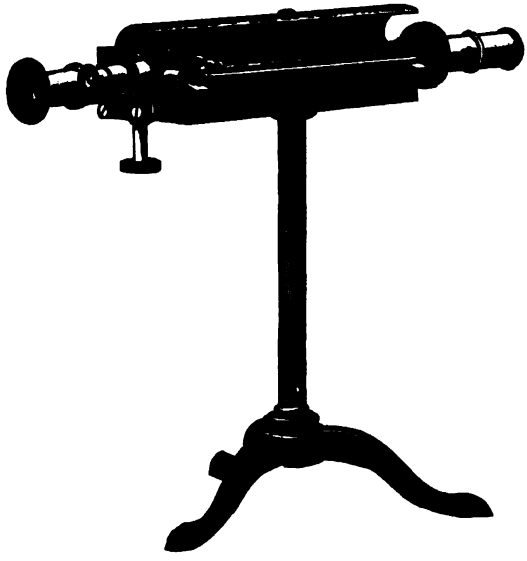


FIG. 29.—Bausch & Lamb Polariscope.

Certain precautions are to be observed in the use of the polariscopic method. Cloudy urines can not be used nor urine so highly colored as to prevent distinct vision. Hence filter the urine if cloudy and, if high colored, decolorize by adding 6 to 8 drops of a 20 per cent. solution of neutral lead acetate to each 10 c.c. of the urine used, filter and make up to the original volume with distilled water.

Albumin should be removed by placing 100 c.c. of urine on the hot water-bath and adding 50 per cent. acetic acid, drop by drop, until the coagulated mass is flocculent, filter quickly, cool, and make up to 100 c.c. with distilled water.

The best temperature of the urine for the polarization determination is from 15° to 20° C. (59° to 68° F.).

☞ The Bausch & Lomb Optical Co., of Rochester and Chicago, supply a polariscope designed especially for urinary work. Figure 29 shows a Bausch & Lomb polariscope.

Laboratory Note.—An objection to the polariscopic method is that any repairs necessary for the instrument must be made by an expert, hence the instrument is not practical in the hands of the general practitioner living at a distance from scientific centers. Moreover, even when new these instruments may not be ready for use when received, but may have to be sent back to the factory for changes.

☞ **Fermentation Methods.**—Fermentation methods are slow, requiring from 6 to 24 hours for completion and lack delicacy, but are on the whole more trustworthy than any of the other methods described. The method of *Roberts* has recently been approved by Koelensmid as readily applied and very trustworthy. This is the most simple method we have and the best suited to the general practitioner: to four ounces of urine in a bottle are added at least one-fourth of a cake of compressed yeast: the whole is shaken well and set aside in a warm, but not hot place for 24 hours. The bottle is loosely stoppered to allow escape of gas, but to prevent evaporation. It is usually recommended that a companion bottle without yeast and tightly stoppered also be set aside at the same temperature. At the end of 24 hours the specific gravity of both samples is taken and the difference in specific gravity indicates sugar in grains per fluidounce. Thus if the specific gravity of the unfermented sample were 1037 and that of the fermented 1010, the urine contains 17 grains of sugar per fluidounce of urine. To obtain *per cent. of sugar* multiply grains per ounce by 0.234; thus in the above 17 times 0.234 = 3.978 per cent. of sugar, or clinically speaking, about 4 per cent.

The writer is, however, opposed to the use of the companion

bottle for the reason that most samples of urine containing sugar have already begun to ferment by the time they are received, hence fermentation will progress and perhaps blow out the cork from the bottle. It is better to use the *chemical thermometer.* Take the specific gravity of the urine at 25° C. (77° F.), then ferment it, cool it if necessary, or warm it to 25° C., and take the specific gravity again. The difference shows sugar in quantity as above.

If the urine is alkaline it should be made acid with tartaric acid.

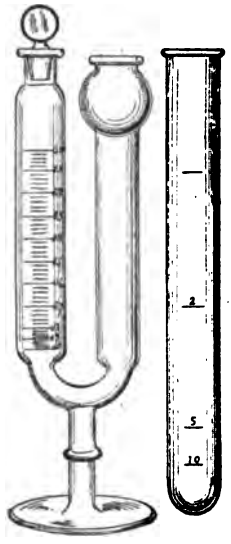


FIG. 30.—Lohnstein's Saccharimeter.

Dr. Heinrich Stern, of New York, has devised an apparatus, the *urine-glucosometer,* for the application of the Roberts method, which is convenient. A less convenient apparatus is J. Schütz's *areosaccharometer* which, however, is said to be more accurate than the Einhorn instrument.

For clinical purposes, however, extreme accuracy is unnecessary. In a general way it is merely necessary to know whether the patient's urine contains less sugar from time to time, or not.

If the percentage is greater than three it is altogether too large, from two to three medium, but if around one or below one is small.

Another apparatus for the purpose of fermentation is that of *Lohnstein* (Fig. 30), designed for greater accuracy. It consists of a U-shaped tube with a long graduated arm closed with a glass stopper and another arm with an open bulb-shaped top

Mercury is poured into the bulb to the zero mark, a measured amount of urine over the mercury, a little yeast added and the glass stopper inserted. The latter is weighted with lead and should be well-greased to prevent escape of gas generated. As the gas is generated, the mercury rises and from the level of the mercury is read off the per cent. of glucose.

The temperature should be from 20° to 35° C. (68°-95° F.). Errors with the use of this instrument are said to occur when albumin is present and according to the dilution of the urine.

☞ **The Einhorn saccharimeter** (Fig. 27) indicates the quantity of sugar and has been described under qualitative testing. The great practical objection to this instrument is the necessity for taking time to dilute the urine so that it shall contain 1 per cent. or less of sugar in the amount poured into the saccharimeter. Compared with Roberts' method the author usually (not always) finds the Einhorn figures higher.

Boston advises diluting the urine for the Roberts' method as in the case when the Einhorn instrument is used.

As to the amount of yeast necessary the writer finds that one-half of one cake is necessary for fermenting 120 c.c. (4 fluid-ounces) of urine in Roberts' method, but only 1 gramme per 10 c.c. of urine in the Einhorn instrument.

The Saccharascope.—This instrument consists of two chambers or receivers, one to contain the fermenting solution, the other to collect and measure the gas evolved in the fermentation. The fermenting chamber is filled to the mark with the urine previously mixed with one gramme (15 grains) of compressed yeast, and a compensation tablet is added, which will evolve just carbonic acid enough to saturate the liquid, so that all the gas that is collected will represent sugar previously present in the urine.

The measuring chamber is filled with water, on which floats a layer of oil to prevent absorption of the gas, the upper surface of the oil being brought exactly to the zero mark. The stoppers are then inserted securely (*any leakage will vitiate the result, of course*) and the apparatus set by in a place where the temperature will be as nearly as possible 80° F., a suitable container being provided to receive the overflow of water. At the end of twenty-four hours, fermentation should be complete, and the volume of gas collected will indicate directly, by the graduated

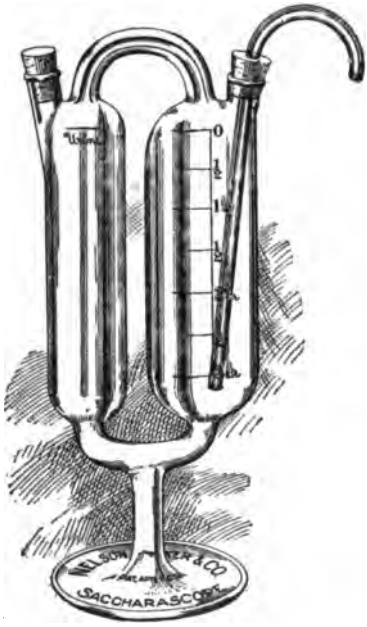


FIG. 31.—The Nelson, Baker & Co. Saccharoscope.

scale of the instrument, the proportion of sugar present. If a preliminary experiment has indicated more than 6.38 grammes of sugar per liter (three grains of sugar to the ounce) it will be necessary to dilute the urine accordingly before testing it, if the exact quantity is to be determined.

The following table converts the indications of the saccharascope directly into percentages, assuming for the urine a uniform specific gravity of 1.020, which is near enough to the truth for all practical purposes:

TABLE.

To convert grains per fluidounce to percentage:

Grains per fl. oz.	Per cent. (approx.)	Grains per fl. oz.	Per cent. (approx.)
1	0.22	14	3.01
2	0.43	15	3.23
3	0.65	16	3.44
4	0.86	17	3.66
5	1.08	18	3.87
6	1.29	19	4.09
7	1.51	20	4.30
8	1.72	21	4.52
9	1.94	22	4.73
10	2.15	23	4.95
11	2.37	24	5.16
12	2.58	25	5.38
13	2.80		

The apparatus and tablets are supplied by Nelson, Baker & Co., Detroit and Kansas City. (Fig. 31.)

Ready Method for Determining the Quantity of Albumin or Sugar.—An ingenious tube is made by a Des Moines chemical company in which the Esbach test for albumin, and the Haines' test for sugar or other cupric test, can be made quantitatively, the latter by boiling the reagent, adding the urine until the reagent is decolorized, then measuring the amount of urine used and calculating the percentage of sugar, which is read off from figures on the tube.

CHAPTER XXIII.

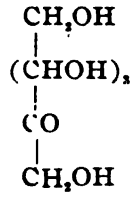
CARBOHYDRATES NOT DEXTROSE. THE CAMMIDGE REACTION.

Carbohydrates not dextrose in urine;—lactose, levulose, etc.
Levulose:—chemistry, structural formula, and significance; "alimentary levulosuria."
Tests for levulose: polarization; Seliwanoff's; Borchardt's; phenylhydrazine.
Galactose;—constitution and tests:—Tollen's test; Barfoed's test; spectroscopic identification.
Laiose: properties and detection.
Honey in urine: melituria.
Pentoses: chemistry, physiology, and significance.
Tests for pentoses:—reduction test; Tollen's orcin test; method of Külz and Vogel; polarization; phenylhydrazine.
Lactose; mistaken for glucose; physiology; occurrence during lactation.
Tests for lactose:—reduction tests; negative tests; mucic acid test; Rübner's test; polarization; isolation.
Sucrose:—accidental occurrence and detection.
Maltose and isomaltose:—properties and detection.
Goldschmiedt's reaction.
Glycerine:—the Cammidge reaction and its clinical significance.
Starch:—microscopic identification.
Glycogen:—isolation and detection.
Clinical method of differentiating the sugars—Newmann's orcin test.

In addition to dextrose other carbohydrates may occur in urine: Lactose, galactose, levulose, fructose, laiose, pentoses, inosite, glycogen or erythrodextrine, maltose, isomaltose, animal gum, saccharose, and starch itself are among the carbohydrates claimed to be found by various observers. Glycuronic acid combinations and alkapton bodies which reduce the copper tests for sugar have already been considered, as also has inosite. The various carbohydrates will be considered in the usual order of classification, viz., (a) monosaccharides: hexoses, pentoses; (b) disaccharides: maltose, lactose sucrose; (c) polysaccharides: starch, glycogen,

dextrine. The hexoses not yet considered are levulose and galactose. Laiose resembles levulose and will be considered among the hexoses.

LEVULOSE.



Chemistry.—Levulose (Fructose), $C_6H_{12}O_6$, is a monosaccharide of the hexose group. Its reducing power is somewhat weaker than that of dextrose. It rotates the plane of polarized light to the left.

Clinical Significance.—In diabetes mellitus levulose occurs along with dextrose, but in rare cases levulose alone may occur. In cases not diabetic but hepatic, ingestion of 100 grammes of levulose may be followed by levulosuria, hence the term "alimentary levulosuria" used by some clinicians as a test for functional diseases of the liver. According to von Halasz, in exceptional cases only 100 grammes of levulose will provoke levulosuria when the liver function is normal. Alimentary levulosuria indicates diffuse and serious liver trouble, and is especially marked in advanced cirrhosis. It does not, however, occur, as a rule, in icterus, nor in secondary metastatic tumors of the liver, nor in circumscribed affections causing limited parenchymatous involvement, as echinococcus, gall bladder diseases, hyperemia, etc.

Tests: 1. *Polarization* is the best test, since levulose rotates to the left. The urine must, however, first be tested for glycuronates, beta-oxybutyric acid, acetone, cystine, and albumin, all of which rotate to the left. When the polariscopic determination of dextrose shows a smaller percentage than titration methods (Fehling's, Purdy's) levulose is probably present along with dextrose. Fermentation removes levulose (from urine rotating to the left), but not the other substances mentioned above.

2. The chemical tests for dextrose already considered are also

affected by levulose. In addition there are certain tests with resorcin, as follows:

3. **Sewilanoff's Reaction.**—To 5 cc. of a solution of 0.05 gm. resorcin in 100 cc. of diluted hydrochloric acid (1 volume of acid to 2 of water) add a few drops of the suspected urine and boil. A red color with separation of a red precipitate indicates levulose. The precipitate is soluble in alcohol, to which it imparts a red color. Too long boiling interferes with this test by showing dextrose. Levulose includes fructose in this test.

4. *Rosin's modification of the Sewilanoff reaction* is as follows: Heat the urine with an equal bulk of hydrochloric acid and a few granules of resorcin; if a red color is obtained, cool and neutralize with sodium carbonate. An orange alkaline fluid results. Shake this with amyl alcohol, which extracts a red color showing a slight shade of yellow and a green fluorescence. On addition of a few drops of absolute ethyl alcohol the color becomes pure rose red.

Dilute solutions show a single band of green from the E line to b. In more concentrated ones this band is darker and extends somewhat over the two lines mentioned, while a second very faint band appears in the blue near F. The addition of alcohol increases the sharpness of the band in green. This reaction and spectrum is given only by ketoses and by the hydrochloride of glucosamine, by the latter only very faintly. The absorption spectrum permits a differentiation between the ketoses and other substances.

5. **Borchardt's Reaction.**—To 5 cc. of urine add an equal volume of 25 per cent. hydrochloric acid and a few crystals of resorcin. Boil, and when a red color appears cool under the hydrant and transfer to an evaporating dish or beaker. Make mixture slightly alkaline with fragments of solid potassium hydroxide, pour back into a test-tube, add 2 to 3 cc. of acetic ether and shake vigorously. A yellow color imparted to the ether indicates levulose. The original color ranges from pale-yellow to red-bronze. This test is very delicate, indicating 0.05 per cent. even when dextrose is present also. Spectroscopically a band is obtained between b and F (green). Borchardt's test is interfered

with by a number of substances, as nitrites, indican, urorosein, santonin, rhubarb. Indican imparts a blue color to the acetic ether, hence if in great excess must be removed by shaking the urine with equal parts of Obermayer's reagent and chloroform. The supernatant urine is decanted off from the chloroform extract and tested for levulose, after previously diluting it with one-third water. Nitrites may be removed by acidulating with acetic acid and boiling for one minute. Large amounts of urorosein should be removed by adding to the urine an equal volume of 25 per cent. hydrochloric acid and shaking two or three times with amyl alcohol.

6. **The Phenylhydrazine test** is the same as with dextrose. Neuberg and Strauss claim to establish the presence of levulose definitely by transformation of it into the crystalline methylphenylosazone. The levulose is precipitated with methyl-phenylhydrazine, as follows:

Titrate a measured quantity of urine with Fehling's solution and add for each gramme of sugar obtained two grammes of methylphenylhydrazine. Let stand several hours and filter, if a precipitate is noticed. Mix the filtrate with 50 per cent. acetic acid using of the acid a volume equal to that of the methylphenylhydrazine used and add enough alcohol to make a clear solution. Heat the mixture from 3 to 5 minutes on a water bath or in an incubator for 24 hours at 40° C. (104° F.), the latter preferable. If large amounts of levulose are present, the osazone is seen in crystals, especially after the addition of a few drops of water. If the amount of levulose is small, an oily liquid only is seen, but this will show crystals if rubbed well with a glass rod. Purify the crystals by dissolving them in hot water to which a little pyridine has been added. Add a little bone charcoal, filter, evaporate to small bulk, and during the evaporation the osazone appears as yellow delicate needles, melting at 158°-160° C. (317°-320° F.). Two decigrammes of methylphenylosazone in 4 cc. pyridin and 6 cc. absolute alcohol rotate to the right 1° 40′ in the 10 cm. tube.

Laboratory Note.—In the case of urine which yields the Sewilanoff reaction, if titration indicates by the Fehling method 6.8

per cent. and polarization 5.9 per cent., and if, after fermentation, the Sewilanoff reaction is negative, dextrose less than 0.1 per cent. by titration, with polarization zero, the conclusion is that the urine contained the original per cent. of dextrose shown by polarization, namely, 5.9 per cent., while the amount of levulose is 0.9 per cent., *i. e.*, 6.8 minus 5.9.

GALACTOSE.

This substance differs from lactose in being a monosaccharide like dextrose, $C_6H_{12}O_6$, while lactose is a disaccharide, $C_{12}H_{22}O_{11}$. Like dextrose it is fermentable and dextrorotatory. Galactose occurs especially in the urine of nursing infants with deranged digestion. Clinically it is, therefore, important to distinguish it from dextrose for fear of the erroneous diagnosis of diabetes mellitus.

Tests.—Galactose, like dextrose, reduces the copper salts. It may, however, be differentiated from dextrose by the mucic acid test. (See Lactose.) It may also be differentiated from dextrose by Tollens' test.

2. **Tollens' Test.**—Add a little phloroglucin to a mixture of equal volumes of urine and hydrochloric acid and heat the whole on a water-bath. Galactose gives a red color as do also glycuronates and pentoses.

3. To distinguish galactose from lactose a positive Barfoed's test may be sufficient. Galactose is fermentable, lactose not.

4. To distinguish galactose from glycuronates and pentoses the spectroscope may be used, which shows no absorption bands in the case of galactose.

Tollens' test for glycuronates previously given may be used by those not familiar with the spectroscope.

LAIOSE.

This substance named after Leo, who discovered it in 1887, has the same empirical formula as dextrose and levulose, $C_6H_{12}O_6$, and is occasionally found in severe cases of diabetes mellitus. It lacks the sweet taste of the sugars and is amorphous and unfermentable. Its taste is salty and it reduces metallic salts

only after long boiling. It combines with phenylhydrazine, giving an oily compound. It resembles levulose in being left-rotatory. Samples of urine containing it show, as in the case of levulose, more sugar by titration than by polarization; as much as 1.2 to 1.8 excess may occur. Urines in which the titration and polarization tests agree contain no laiose. Whenever the titration method shows excess over the polarization and when, at the same time Sewilanoff's reaction is absent, laiose may be suspected.

HONEY IN THE URINE.

Malingerers sometimes add this substance to the urine, causing a high specific gravity and positive reaction with copper tests. Such urine may give but very few phenylhydrazine crystals, ferments very slowly, and may be dextrorotatory, if a trace of glucose or inverted cane-sugar be present. To detect it with certainty concentrate the urine by boiling, boil 20 to 40 minutes with dilute hydrochloric acid, neutralize with sodium bicarbonate, and then obtain tests for a mixture of dextrose and levulose with a polarization of zero. The term *melituria* is applied to this condition.

PENTOSES.

Sugars with a chain of five carbon atoms, ($C_5H_{10}O_5$).

Pentoses occur in the urine either as a temporary or a persistent phenomenon. In the first case the condition is alimentary, in the second bears no relation to diet. The pentoses found in urine are arabinose, xylose, and rhamnose.

Chemistry.—They are unfermentable and on heating with dilute mineral acids yield furfurol without levulinic acid.

Physiology.—Pentoses are products of the hydrolysis of the other carbohydrates in the body and have been thought to be formed from the carbohydrate nucleus in the neucleo-protein molecule of certain organs, viz., the pancreas, thyroid, thymus, brain, spleen and liver, but Neuberg denies this. They are common in certain articles of food, as prunes, cherries, grapes, plums and fruit juices; they also occur in beer. They pass when pure more easily into the urine than other carbohydrates are able to

do. The power to assimilate them varies with different individuals.

Clinical Significance.—When only temporarily present their origin is alimentary and referable to ingestion of the articles mentioned above, *i. e.*, fruits, fruit syrups, malt liquors, etc. Persistently present the condition is known as "idiopathic," *i. e.*, without known cause. They also occur in the urine in diabetes mellitus, especially in the severest cases. They have been found in the urine of persons addicted to the use of morphine, but seem to bear no relation to the addiction.

Tests.—1. Pentoses reduce the alkaline copper solutions. The reduction of Haines' test liquid is most likely to occur suddenly after the mixture has cooled. Nylander's test shows a gray precipitate. The fermentation test with the Einhorn instrument is negative. Arabinose is optically inactive, the other two dextrorotatory.

2. **Tollens' Reaction.**—Same as for galactose, which see.

3. **Orcin Test.**—Add a small amount of orcin to equal volumes of urine and hydrochloric acid (the latter of specific gravity 1.19) and boil. The color changes from red through reddish blue to green. When it has become green, it should be cooled until only warm, then shaken in a separatory funnel with a little amyl alcohol and the extract examined with the spectroscope. An absorption band between C and D will be observed.

The urine should first be decolorized with animal charcoal. The reddish color may be very transitory or absent altogether.

4. **Bial's Test.**—Ferment the urine and then test for pentoses as follows:—make up Bial's reagent:—500 c.c. of 30 per cent. hydrochloric acid, 1 gramme of orcin, and 25 drops of the liquor ferri sesquichloridi of the German Pharmacopœia (10 per cent.). Boil 5 cc. of this reagent in a test-tube, remove from the flame, and add not more than 1 cc. of the urine. A green color should appear at once and not enough heat is used to show the glycuronates. In some cases when hexoses (dextrose, levulose, galactose) are present, pentoses disapear during fermentation, probably through the agency of yeast bacteria. If hexoses are present the following is recommended:—

5. **Method of Külz and Vogel.**—From 1.6 to 3.2 litres of urine are used. For each 100 gms. of glucose are added 200 gms. of phenylhydrazine plus 100 gms. of glacial acetic acid. The urine is then heated to a water-bath for an hour and a half, cooled, and filtered. The filtrate is again heated on the bath for one and a half hours and filtered. The combined precipitates are well washed with cold water and digested in water at 60° C. (140° F.), which dissolves the pentosazone. Glucosazone is dissolved only on heating to boiling. One liter of water per 100 gms. of sugar is used, and the digestion continued twelve hours. This is repeated fifteen times. The hot extracts are filtered, then allowed to cool, and the pentosazone will separate. This is repurified, using less water till the melting point is constant.

Polarization distinguishes xylose from arabinose in an alcoholic solution. Xylosazone is levorotatory, while arabinosazone is optically negative, except immediately after fermentation, when it is dextrorotatory.

6. **Phenylhydrazine Test.**—Salkowski's method is to take from 200 to 500 cc. of urine and to each 100 cc. are added 2.5 grammes of phenylhydrazine dissolved in sufficient acetic acid to acidify it. The mixture is heated to boiling, then cooled in water for an hour and a quarter. The crystals of phenylpentosazone are soluble in water at 60° C. (140° F.) and melt at 158° C. (316.4° F.).

7. **Quantitative Method of Neuberg and Wolgemuth.**—Titrate approximately with Fehling's solution, and if less than one per cent. appears to be present, concentrate the urine in vacuo, until about one per cent. is found. Mix 100 cc. of the urine or concentrated urine with 2 drops of 30 per cent. acetic acid and evaporate on the water bath to 40 cc.; add 40 cc. of hot 96 per cent. alcohol and cool. Then let stand 2 hours, filter, wash the residue carefully with 40 cc. of 50 per cent. alcohol. Add 1.25 grammes of pure diphenylhydrazine to the filtrate, warm the mixture one-half hour on the water bath and replace the evaporated alcohol. Let stand 24 hours in a cold place, collect the crystals in a Gooch filter, using the filtrate to collect crystals. Wash the contents of the filter with 30 cc. of 30 per cent. alcohol and dry all at 80° C. to a constant weight. The amount of arabinose present is calculated by multiplying the quantity of osazone obtained by 0.4747.

LACTOSE.

This substance like galactose is devoid of clinical interest but is of considerable chemical importance in that it is so frequently mistaken for dextrose and the erroneous diagnosis of diabetes mellitus made. Lactose is a disaccharide.

☞ **Physiology.**—Lactose is found in the urine of those who have been on a milk diet for a long time, or who have taken 100 grammes or more of it at a dose. In practice we find it in the urine of nearly all lactating women, but usually in small amount, 0.013 to 0.438 per cent. Occasionally, however, it may be as high as 2 or 3 per cent., reaching a maximum from the second to fourth day after delivery. Stasis in the mammary glands is the cause of the appearance of it in the urine. It is seen in cases of mastitis and in women who have weaned their children early. Lactosuria lasts usually but a few days, but in some cases continues for a week. Either superabundance of milk or suppression of lactation causes it. Its appearance in the urine is referable to absorption, since once in the circulation it can not be inverted, as the body is unable to invert disaccharides to monosaccharides. Administration of large amounts of dextrose (150 grammes) in one dose to nursing women may cause lactosuria.

Tests.—Lactose responds to the copper tests and to Nylander's test. When ten drops of urine containing lactose are added drop by drop to one fluidrachm of Haines' solution with boiling after each drop, reduction does not usually take place until five drops or more have been added, owing to the fact that usually but a small amount of lactose is present, and the reaction is slower with lactose than with dextrose.

☞ 1. *To demonstrate clinically the presence of lactose* a positive reaction with Haines' test as above, a positive reaction with Nylander's test, and a negative fermentation test made upon the boiled and cooled urine with the Einhorn instrument are sufficient, granted that it is known that the patient is a lactating woman with no symptoms suggesting diabetes mellitus.

In addition the phenylhydrazine test is unsatisfactory and Bar-

foed's test with cupric acetate and acetic acid is negative. (Barfoed's solution is made by dissolving 4.5 grammes of neutral cupric acetate crystals in 100 cc. of water and adding 0.12 cc. of 50 per cent. acetic acid to it.) The test is made by boiling 5 cc. of the solution and adding urine drop by drop as in Haines' test, a red precipitate being positive either during boiling or after cooling. All monosaccharides respond to this test and disaccharides after long boiling. Another way of preparing Barfoed's solution is to dissolve one part of cupric acetate in 15 parts of water; to 200 cc. of this solution add 5 cc. of acetic acid containing 38 per cent. of glacial acetic acid.

2. **Mucic Acid Test.**—Since nitric acid converts lactose into mucic acid by oxidation, this fact may be utilized in a test, as follows: The specific gravity of the urine is taken and if under 1020, 100 c.c. of it are measured out and 20 c.c. of strong nitric acid added. If the specific gravity of the urine is above 1020, then from 25 to 35 cc. of the acid are added.

The mixture is evaporated on the water bath down to the volume of nitric acid added. A fine white precipitate of mucic acid appears, either when hot or after cooling, or after standing several hours.

3. **Rübner's Test.**—To 10 c.c. urine add 3 grammes of lead acetate and filter. Heat the filtrate three or four minutes until a brown color appears. Then add ammonia and heat again when, if lactose is present, a brick red color with cherry red precipitate appears and the supernatant urine is decolorized. Urine over 1020 in specific gravity should be diluted with equal parts of water. The test is not delicate enough to detect the small quantities of lactose usually present in urine.

4. **Isolation.**—The method advised by F. Hofmeister is to precipitate the lactose directly with acetate of lead and ammonia. C. E. Simon gives it as follows: the collected urine of twenty-four hours is precipitated with lead subacetate and filtered. After washing with water the filtrate and washings are mixed and treated with ammonia. The resulting precipitate is filtered off and the filtrate again precipitated with lead subacetate and ammonia, and so on until the final filtrate is optically inactive. The

precipitates, with the exception of the first, are then mixed, washed with water, decomposed with hydrogen sulphide and filtered. In the filtrate the excess of hydrogen sulphide is removed by a current of air and freed from any acids that have been liberated by shaking with argentic oxide. The mixture is filtered, freed from soluble silver with hydrogen sulphide, treated with barium carbonate, and concentrated to a small volume; 90 per cent. alcohol is then added, which causes the formation of a flocculent precipitate. This is filtered off. The filtrate is placed in the desiccator, when, on standing, crystals of lactose gradually separate out. These may be purified by recrystallization, decolorization with animal charcoal and extraction with 60-70 per cent. alcohol.

SUCROSE (CANE SUGAR).

This disaccharide substance may occur in the urine of persons who have been eating a large amount of cane sugar, but it may quite frequently occur accidentally in the urine when containers are used in which syrup has previously been present. Insane or hysterical persons may sometimes add it to their urine, and medical students may think it "funny" to add it to the urine of a colleague. No reduction of the copper tests takes place, hence it may be suspected in a urine of high specific gravity but of normal volume which fails to yield a copious colored precipitate with Haines' or Fehling's tests. Such urine rotates to the right, but after boiling with dilute acid rotates to the left. An excess of it may cause a slight greenish cloudy precipitate with Haines' solution, possibly from some impurity, if of accidental occurrence.

MALTOSE AND ISOMALTOSE.

These substances are disaccharides like sucrose and lactose. It is claimed that isomaltose occurs in normal urine in which it is to be demonstrated as a benzoate.

Maltose occurs occasionally in small and variable amount in diabetes mellitus and according to Jaksch in malignant tumors. It has also been found in a supposed case of pancreatic disease. Isomaltose forms an osazone with phenylhydrazine, having very

fine crystals which melt between 150° C. (302° and 307° F.). It is said to be of no clinical importance.

Isomaltose is non-fermentable, dextrorotatory and reduces copper and bismuth. It is precipitated with other carbohydrates by the Baumann method with benzoyl chloride.

Maltose forms an osazone with phenylhydrazine which may be identified by its melting point about 16° C. lower than that of dextrosazone crystals. The osazone occurs usually in yellowish, coarse sheaves rather than needles. The maltosazone contains 10.6 per cent. of nitrogen. Two c.gm. of the osazone in a mixture of 4 cc. pyridine and 6 cc. absolute alcohol rotate to the right 1° 30′.

GLYCOGEN (ERYTHRODEXTRINE).

This substance may occur in the urine of diabetes mellitus after the dextrose disappears or diminishes. In such cases the urine reduces copper salts only after long boiling with Trommer's test, showing first green, then yellow, then brown. Isolated from the urine a white tasteless powder is obtained, which is soluble in water, reduces copper salts slowly, but gives a brown color with solution of iodine. Isolation can be made by treating the urine with five volumes of alcohol and washing the precipitate on a filter with alcohol repeatedly. It is regarded by some authorities as identical with the animal gum of Landwehr. Urines which after fermentation, on prolonged boiling, again reduce the cupric tests may be suspected of containing glycogen.

STARCH.

Starch itself has been found in the urine of infants. The granules are to be recognized by the microscope by their concentric layers and hilum. Starch powder may be used as a local application and hence may be found in the urine of adults also. It is to be recognized in the same manner. Potato starch granules are large and have concentric layers, but corn-starch, which is more common, shows in addition an indentation (hilum) near the centre of the granule, while in potato starch the hilum is not in the center. (See Sediments.)

☞ **A Clinical Method for the Identification of Sugars** in

Urine. When, by the use of the copper tests, a doubtful reaction occurs, viz., a slight yellowish or greenish-yellow turbidity only after much urine, relatively speaking, has been added or not until the mixture cools, the question is always the following: "Is it dextrose (glucose)?" If the previous history of the patient is unmistakably that of diabetes mellitus no further consideration of the condition is necessary, but if no previous history of diabetes can be obtained, the question is one of much importance. The author uses the Einhorn saccharimeter in doubtful cases as follows:—10 cc. of the suspected urine are boiled, cooled, one gramme of washed yeast added and the whole, after stirring, poured into the Einhorn instrument, and allowed to stand together with a companion tube containing the same yeast and normal urine until the next day in a warm room. Absence of fermentation excludes dextrose, levulose, galactose and probably laiose indirectly, and points to lactose, glycuronates, pentoses, maltose, isomaltose, excess of normal constituents, presence of alkapton bodies, or extraneous substances as the original cause of the reduction of the copper test.

If the patient is a lactating woman the substance is in all probability *lactose*, dextrose being excluded by the absence of fermentation. The original urine may be tested with the mucic acid test (positive) and Barfoed's test (negative). Rübner's test may also be tried, but a negative result with it does not exclude lactose, so far as the writer's experience goes. If the patient has been taking drugs like chloral, or if chemicals as chloroform have been added to preserve the urine, the reduction is probably due to *glycuronates*, in which case the simplest procedure is to obtain another sample from which the drugs or chemicals are excluded and to test again, a negative result confirming the suspicion of presence of glycuronates of the previous occasions; or the copper reducing urine can be tested with B. Tollens' naptho-resorcin test for glycuronates as per direction in Chapter XII. If lactose and glycuronates are absent and, especially, if the copper solution is reduced *suddenly*, as the urine cools, suspect presence of *pentoses*, particularly if the patient has been eating heartily of cherries, plums, or other fruits or partaking of fruit

juices or drinking beer freely. If pentoses are present, Nylander's test gives a grayish precipitate, and Bial's test gives a green color immediately after boiling.

Maltose occurs chiefly in diabetes mellitus, hence is of no clinical importance when this disease is unmistakably present. A doubtful reaction with the copper tests in a case of malignant tumor or of pancreatic disease may possibly be due to it. If thought necessary to identify it, its osazone with phenylhydrazine should be precipitated, when the microscope will show yellowish, coarse sheaves rather than the finer needles seen in the case of the other sugars, and the melting point of the crystals (about 16° C. lower than dextrosazone) may serve to identify them. Maltose is dextrorotatory. Two cg. of the maltosazone in a mixture of 4 c.c. pyridin and 6 c.c. absolute alcohol rotate to the right 1° 30′.

Isomaltose forms an osazone with phenylhydrazine showing very fine crystals which melt between 150° and 153° C. (302° and 307° F.). Isomaltose is dextrorotatory.

Alkapton, by virtue of its homogentisic acid, reduces the copper tests. Urine containing alkapton may be easily recognized by the disappearing dark green color yielded with ferric chloride solution, and by the writer's test (brown foam and brown color when floated on the solution of hypobromite used for the urea determination). Such urine blackens from above downward when exposed to the air and also on the addition of alkalies.

In the absence of identification of the above-named constituents the presence of *accidental constituents* is to be suspected. To be sure of this, information must be had of the previous contents of the bottle in which the urine was supplied. If this bottle had previously contained foods, drugs, chemicals or perfumery, the chances are that the reduction was due to a small portion of these substances left over. Test-tubes used for tests where acetic acid has been employed, if not well cleaned, may be responsible for the reduction, since even one drop of 20 per cent. acetic acid may cause a turbidity when boiled with one fluidrachm of Haines' solution.

When now fermentation is positive, shown by a decided lower-

ing of the fluid in Einhorn's instrument in the case of the suspected urine, while at the same time there is none in the case of the companion-tube holding the normal urine, the presence of dextrose, levulose, or galactose is to be inferred, together with the possibility of laiose as a concomitant.

If the patient is a nursing infant it is of great importance to identify *galactose* for fear of a mistake in the diagnosis. To identity galactose, the original urine is tested with E. Tollens' phloroglucin test, and also the mucic acid test, both of which differentiate it from dextrose.

If galactose is absent, the remaining sugars are dextrose, levulose, and laiose. Polarization is the best method for differentiating dextrose from levulose, but since this procedure is beyond the reach of the general practitioner, the writer advises the alimentary test as follows:—cause the patient on an empty stomach, preferably at noon, to eat a carbohydrate luncheon including a quarter pound or more of cheap candy or glucose syrup. Test the urine voided two hours or more after luncheon for dextrose with the copper test and a marked positive result indicates that the patient is intolerant of dextrose, hence the inference is that the original urine of the previous occasion contained dextrose. If no result is obtained by feeding as above, repeat the experiment on another day. If the result is still negative, try on another day feeding with 100 grammes (one quarter pound) or more of levulose which can be obtained of E. H. Sargent & Co. and other dealers in chemicals. A positive reaction with the copper tests under such circumstances indicates levulosuria and points to the liver as the seat of the trouble.

Laiose is to be found only so far as known in diabetes mellitus, hence the identification of it for clinical purposes appears at present to be unnecessary. Its presence is to be suspected when, in the absence of a Sewilanoff reaction, titration gives higher figures than polarization.

If the various sugars, including lactose, glycuronates and pentoses are absent, the reduction of the copper solution in the absence of fermentation, alkapton, and extraneous substances is likely to be due to excess of *urinary normal constituents*. In

such cases the urine is practically always *scanty* in amount and of increased color and specific gravity. This reduction by normal constituents (coloring matter, uric acid, etc.) is fairly common in cases when either the day urine is small in volume compared with the night or vice versa. *The reduction occurs in the case of the smaller volume of urine, but not in the larger.* If Haines' solution is employed, it will usually, in such cases, require eight to ten drops of the urine to precipitate the cuprous oxide, and perhaps then only after cooling, when the precipitate slowly forms. Cause the patient to drink freely of water, test his 24 hours' urine, and absence of the reduction points to excess of normal constituents in the previous test.

Neumann's Orcin Test.—Three cubic centimeters of urine are treated with 10 drops of a 5 per cent. alcoholic orcin solution and 10 cc. of glacial acetic acid. Boil and let cool. After cooling add strong sulphuric acid drop by drop with shaking until 20 drops have been added. *Pentoses* give an olive-green, *glycuronates* a violet, *dextrose* a carmine.

This test may be used in conjunction with or as confirmatory of the fermentation method with the Einhorn saccharimeter.

Guido Goldschmiedt's Alpha-naphthol Test.—This test, described under glycuronates, may be useful in differentiating if the observer has a correct appreciation of shades of color, since in normal urines the reaction is reddish purple, with glucose blue, and with glycuronates distinctly violet. Albumin must be absent, and it is well to rule out nitrates and nitrates with the diphenylamine test.

GLYCERINE (?). THE CAMMIDGE REACTION.

A glycerin-like substance is excreted in the urine in some cases of pancreatic disease, being set free from fat and entering the blood. Much discussion has been provoked by the claims of Cammidge as to the clinical value of the reaction showing it.

H. Kehr claims that the Cammidge reaction should be employed to determine the soundness of the pancreas in cases of gallstone disease. Kerr examined 50 cases of gallstone disease during the months of March and April, 1909. Twenty-five, that is exactly half of the cases, were operated upon. The urine of

thirty-two of the patients was submitted to the pancreas reaction of Cammidge. A positive result was obtained in 25, a negative result in 7 cases. Of the last named 7 cases 4 were operated upon; the pancreas was always found to be healthy and soft. Of the 25 instances in which the reaction was positive, 18 were operated upon. The reaction was misleading in but one case, inasmuch as a carcinoma of the choledochus was found instead of a supposed chronic pancreatitis. The reaction proved, therefore, trustworthy in about ninety per cent. of the cases.

Again, J. Speese and E. H. Goodman find the Cammidge reaction a constant feature in hemorrhagic pancreatitis, in mechanical injuries of the gland (crushing of the tail, partial extirpation), and in total extirpation. In certain cases of the subacute type of pancreatitis the reaction is inconstant. The nature of the phenylhydrazine compound is not definitely established. A positive reaction is indicative of altered carbohydrate metabolism, due to disturbances of the internal secretion of the pancreas.

Cammidge claims that his reaction is positive in all cases of pancreatitis, negative in healthy subjects, in 75 per cent. of cases of cancer, and with very few exceptions in non-pancreatic diseases. He claims to distinguish acute from chronic pancreatitis by the solubility of the osazone crystals in 33 per cent. sulphuric acid; if they require under the microscope a minute or less for solution the case is one of acute pancreatitis; if more than a minute chronic pancreatitis; if five minutes pancreatic cancer.

It is needless to say that these claims have not been accepted in toto.

Smolenski, of St. Petersburg, claims that the Cammidge reaction is due to cane sugar and not therefore necessarily characteristic of pancreatic disease.

Cammidge's Reaction.—The urine examined must be from a twenty-four hours' specimen or from the mixed morning and evening excretion. If albumin is present, the specimen must be faintly acidified, boiled, filtered, and made up to the original bulk with distilled water. If sugar be present, it must be removed by fermentation with yeast, and if the urine is alkaline, it must be made just acid before proceeding.

To 40 c.c. of urine add 2 c.c. of strong hydrochloric acid, place in a small flask with a funnel in the mouth, boil for ten minutes, then cool the flask thoroughly in a stream of water, and make up to 40 c.c. with distilled water.

Add slowly 8 gm. of powdered lead carbonate, allow the mixture to stand for a few minutes, and when the reaction is complete, cool and filter through a close-grained filter-paper until the filtrate is perfectly clear. Ten c.c. of this filtrate are diluted with 20 c.c. of distilled water.

Shake this clear filtrate with 2 gm. of powdered tribasic lead acetate; filter several times, getting as clear a filtrate as possible. Do not use the U. S. P. solution of lead subacetate.

Add 2 gm. powdered sodium sulphate and boil. Cool the flask in a stream of water and filter to make 20 c.c.

To 20 c.c. of the filtrate add 0.8 gm. of phenylhydrazine hydrochloride, 2 gm. of sodium acetate, and 1 c.c. of 50 per cent. acetic acid. Boil on a sand-bath for ten minutes and filter while hot through a small funnel moistened with hot distilled water. If the result is less in volume than 15 c.c. make up to that bulk with hot distilled water, thoroughly mixing the whole.

In case of pancreatic disease, a light yellow flocculent precipitate forms. This must be examined microscopically, and will be found to consist of thread-like crystals arranged in sheaves and bundles. They dissolve in ten to fifteen seconds on being treated with 33 per cent. sulphuric acid. Any precipitate other than the crystalline deposit mentioned is not to be regarded as evidence of a positive reaction.

A control test is carried out the same way, except that the preliminary boiling with HCl is omitted; this, of course, gives crystals if sugar is present, and in that case, the test has to be repeated after fermentation with yeast.

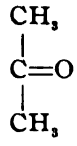
CHAPTER XXIV.

THE ACETONE BODIES.

Structural formula and chemistry of acetone.
Physiology of acetone.
Pathology of acetone; relation to fevers and diabetes mellitus; acidosis.
Tests: Folin's skepticism as to "acetone" tests; Legal-Kelly test; Gunning's; Taylor's; Trommer's; Lieben's; Reynolds-Gunning's; ethylene-diamine test.
Distillate tests for acetone.
Quantitative determination of acetone (Folin's method).
Quantitative determination of acetone (Emerson's method).
Structural formula and chemistry of diacetic acid.
Clinical significance of diacetic acid; relation to diabetes mellitus.
Relation of diacetic acid to the prognosis in diabetes mellitus.
Gerhardt's test for diacetic acid; the older method; the method used by the author; the method of extraction and testing.
The Lipliawsky-Arnold test; assertion of Allard.
Quantitative determination of diacetic acid: the Folin-Hart method for acetone and diacetic acid together; determination of amount of decinormal iodine to use.
The Folin-Hart and Folin-Messinger-Huppert method for determination of diacetic acid.
Structural formula and chemistry of beta-oxybutyric acid.
Clinical significance of beta-oxybutyric acid; relation to diabetes mellitus.
The prognosis of impending coma.
Pathology of beta-oxybutyric acid; origin of the acid in the body; acidosis.
Origin of beta-oxybutyric acid according to C. E. Simon.
Detection: clinical test of Black; Külz's test; polariscopic test.
Quantitative determination of beta-oxybutyric acid: clinical method; Black's method; Shaffer's method; Darmstaeder's method; method of the Strassburg clinic; Bergell's method; method of Boekelman and Bouma.

The substances conveniently classified as acetone bodies are acetone, diacetic acid, and beta-oxybutyric acid.

ACETONE.



Chemistry.—Acetone is dimethyl ketone, $CH_3—CO—CH_3$, a thin, colorless liquid of fruity odor, boiling at about 56° C. (133° F.), and soluble in water, alcohol, and ether. Its specific gravity is about 0.8.

Acetone in the urine arises chiefly from the oxidation of beta-oxybutyric acid. Its immediate mother-substance is diacetic acid. The following equations show the relations of the three substances:

$$\underset{\text{(beta-oxybutyric acid)}}{CH_3.CH(OH).CH_2.COOH} + O = \underset{\text{(diacetic acid)}}{CH_3CO.CH_2.COOH} + \underset{\text{water)}}{H_2O}$$

Finally diacetic acid equals acetone and carbon dioxide.

Physiology.—Acetone is a normal constituent of urine derived from protein or fat decomposition, occurring in quantity per 24 hours of 0.01,—0.03 gramme. It is increased by a protein diet after 48 hours, as, for example, by the continuous administration of the whites of eggs. Alcohol increases it. It is always increased when the carbohydrates in the diet are limited, hence increases during hunger. A large amount of fat ingested (150 grammes) will increase it. Alkalies ingested decrease it. Rectal feeding may increase it.

Pathology.—Acetonuria (the voiding of urine containing acetone in considerable amount) occurs principally in fevers and in diabetes mellitus. Of the fevers, scarlet fever, typhoid, and pneumonia are more likely to be accompanied by acetonuria, though it is known to occur frequently in measles and smallpox. It may also occur in nephritis, phosphorus and chloroform poisoning, severe anemias, derangements of digestion, autointoxication, ether and chloroform anesthesia, cerebral irritations, certain cancers independently of inanition, gastric ulcer, mental diseases, inanition and cachexia, psychoses and lesions of the central nervous system (especially when associated with starvation),

pregnancy, poisoning by certain agents (as phloridzin) extirpation of the pancreas, paresis, melancholia, tabes, after epileptic seizures, in Addison's disease, eclampsia, and the vomiting of pregnancy. It may be present in the breath, causing a fruity odor as when in large amount in the urine.

☞ **Clinically,** we find it most common in the urine of children with fevers and in severe or advanced cases of diabetes mellitus, in which latter condition more than 5 grammes per 24 hours may be found; the quantity increasing toward death and during coma.

Clinical Note.—Idiopathic acidosis occurs according to Sharp in children, the symptoms being sweet breath, drowsiness, vomiting, headaches, thirst, frequency of respiration, and loss of appetite. Acetone and often diacetic acid are present, but no sugar. Recovery may take place in from 24 to 48 hours, but cases may terminate fatally in 8 or 9 days.

Occurrence of Acetonuria in Cases of Infectious Diseases.—According to A. Harris, in the *Lancet*, 1910, in scarlet fever and diphtheria acetone is almost invariably present, even in the mild cases. The more severe the disease, the larger is the amount of acetone in the urine. In a mild case of diphtheria the acetone disappears from the urine on about the seventh day of the disease. The presence of acetonuria seems to possess some diagnostic value in differentiating between diphtheria and scarlet fever on the one hand, and an ordinary sore throat on the other. It is much more constantly present in the former cases. In adults, even in cases of diptheria and scarlet fever, it is not present so constantly as in children. (Abstract in *Archiv. of Diagnosis.*)

Detection.—The physiologist, Folin, is quoted as saying that the tests for acetone in urine show diacetic acid rather than acetone. Nevertheless, if the acetone occurring in commerce be purchased and mixed with normal urine previously negative to acetone tests the mixture will respond to these tests, as will also urine having the strong and well known odor of acetone, which is certainly not that of diacetic acid. Moreover, the ferric chloride test is sometimes positive in urines which are negative to the acetone tests.

A good clinical test is that of **Legal** modified by Kelly: to about 5 c.c. of urine in a test-tube add a few crystals of sodium nitroprussiate and wait until they dissolve, shaking the tube occasionally. Enough of the crystals should be added to color the urine yellow-red. Add an equal volume of strong sodium hydroxide (as, *e. g.*, that used for the urea hypobromite solution), shake and immediately add glacial acetic acid (about one-half volume). When the alkali is added the mixture turns red and on further addition of the glacial acetic acid the red color deepens, if acetone is present in quantity, until almost purple and the foam is of deep red color. In the absence of acetone the color disappears when the acetic acid is added and a green color (due to creatinine) takes its place. It is useless to rely upon this test unless the operator's technique is skillful.

☞ For inexperienced persons the proportions advised by the author's assistant, L. F. Roblee, are more trustworthy as follows:

Dissolve a few crystals of sodium nitroprussiate in 3 c.c. of urine by shaking. Add, in order, 4 drops of glacial acetic acid, 2 c.c. of 40 per cent. sodium hydroxide, and 1 c.c. of glacial acetic acid. Shake quickly and if acetone is present the deep red color and purplish foam will be seen.

The urine in all cases should be freshly voided. In some cases of diabetes mellitus, however, so much acetone is present that the test is positive after the urine had stood several days.

Laboratory Note.—It is said that paracresol gives a reddish-yellow and acetic acid a clear rose with this test, and that to exclude aldehyde it is well to use ammonia instead of sodium hydroxide.

2. **Gunning's Test.**—This may be applied to the urine directly. To 5 c.c. of urine add a few drops of tincture of iodine or Lugol's solution and then ammonia water, until a deep black precipitate forms which later on long standing becomes a yellowish sediment of iodoform. When acetone is present in small amount let the tube stand for 24 hours. The microscope shows flat six-sided yellow tablets or stars having the iodoform odor. If the crystals are imperfect, dissolve them in ether and recrystallize.

Crystals of stellar or triple phosphate may also be present and must not be confused with those of iodoform. The iodoform crystals, if star-shaped, have six processes. If hexagonal, may have six radii.

Laboratory Note.—Lugol's solution is made by dissolving 4 grammes of iodine and 6 of potassium iodide in 100 c.c. of water.

3. **Taylor's Test.**—Taylor has modified Legal's test by omitting acetic acid altogether. He treats the urine with the nitroprussiate and then floats ammonia water on it. Acetone gives a magenta color at the contact, but normal urine an orange-red. The objection to this test is the inability of inexperienced persons to distinguish somewhat similar colors.

4. **Fromme's Test.**—To 10 c.c. of urine add 1 gramme of potassium hydroxide and immediately follow with 10 drops of a 10 per cent. solution in alcohol of salicylaldehyd. (Acid salicylous, Merck.) The mixture is then gently heated to 70° C. 158° F.) and, if acetone is present, a deep-red color appears at the bottom of the tube at the line of contact.

5. **Lieben's Test.**—This is also an iodoform test in which, to 5 c.c. of urine, potassium hydroxide solution is added until alkaline and from 1-2 c.c. of iodine solution, drop by drop. The objection to this test is the fact that if the urine has undergone fermentation the alcohol will yield iodoform and, if aldehyde, from too long distillation of the urine, is present, this also will yield iodoform.

6. **Reynolds-Gunning Test.**—To 5 c.c. of urine add a few drops of mercuric chloride solution, render alkaline with potassium hydroxide and add an equal volume of 95 per cent. alcohol. Shake well until most of the mercuric oxide is dissolved and filter. Acidify faintly the clear filtrate with hydrochloric acid and float ammonium sulphide upon the solution. At the zone of contact a grayish ring of precipitated mercuric sulphide will form. Aldehyde (from too long distillation) yields the same reaction. The principle of the test is the solubility of mercuric oxide in acetone.

7. **Ethylene-Diamine Test.**—Place in a test-tube, which has been thoroughly cleaned with hot water, about five cubic centi-

meters of urine and add a few crystals of sodium nitroprussiate (nitroprusside). Shake until dissolved. Then add equal parts of urine to be tested and mix well. Overlay the mixture with a few drops of 10 per cent. ethylene-diamine-hydrate solution. A pink or ruby-red at the juncture indicates acetone. A faint white cloud is due to the reagent.

8. **Distillate Tests.**—The various authorities insist upon distilling the urine before giving a negative report on acetone, yet the author finds Kelly's clinical test described above to yield positive results in diabetes mellitus in hundreds of cases when properly employed.

In order to distil the urine use a glass retort holding about a liter, into which about 500 c.c. of urine are poured and a little phosphoric acid (1 gramme per liter) added to prevent evolution of gases. Boil the mixture and collect the first 30 c.c. of distillate, which will contain all of the acetone. To this distillate all the tests mentioned above may be applied with greater certainty, it is claimed, than when the urine itself is used. Another method is to place 100 to 250 c.c. of the urine in a retort, render it acid with acetic acid, distil one-third of it, add 5 drops of 10 per cent. hydrochloric acid to the distillate and redistil about one-half the volume. Fig. 32 shows an apparatus for distillation.

Quantitative Determination.—1. The method of *Folin* is as follows: use the ammonia apparatus of Folin, introduce 25 c.c. of urine into the aerometer cylinder and add 10 drops of 10 per cent. phosphoric acid or 0.2-0.3 gramme of oxalic acid, 8-10 grammes of sodium chloride in saturated solution of which acetone is insoluble, and a little petroleum. Procure Folin's improved absorption tube and into a Folin ammonia absorption flask provided with this tube introduce 150 c.c. of water, 10 c.c. of a 40 per cent. solution of potassium hydroxide and an excess of decinormal iodine solution. Connect the flask with the aerometer cylinder, attach an air pump and cause an air current, not quite so rapid as in the case of ammonia, to be drawn through the solution for 25 minutes. Iodoform collects in the absorption flask. Add 10 c.c. of strong hydrochloric acid to the con-

tents of the flask and titrate the excess of iodine with decinormal thiosulphate solution and starch indicator.

Results are calculated as follows: the sodium thiosulphate solution is added until a light yellow color is observed, when a few c.c. of starch paste are added and more of the thiosulphate until the blue color has disappeared. Subtract the number of c.c. of thiosulphate solution used from the number of c.c. of iodine solution employed. Since 1 c.c. of the iodine is equivalent

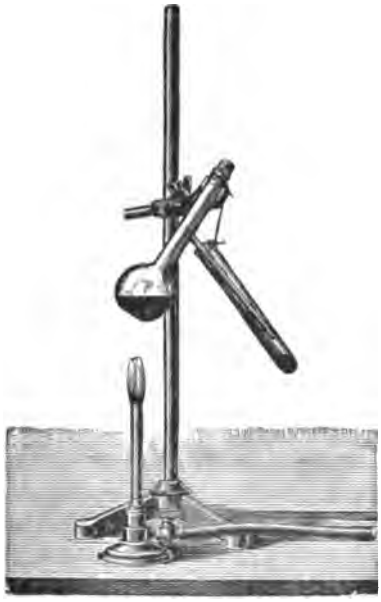


FIG. 32.—Apparatus for Distillation (Sahli).

to 0.967 milligramme of acetone and since 1 c.c. of the thiosulphate solution is equivalent to ' c.c. of the iodine solution, the difference obtained above multiplied by 0.967 gives us the amount of acetone in milligrammes in the 25 c.c. of urine employed.

To calculate the ammonia and acetone at the same time in cases of acidosis, Folin suggests the following: arrange the ammonia apparatus as usual (Chapter VI.) and to the aerometer

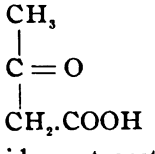
attach the acetone apparatus set up as described above. Regulate the air current with special reference to the determination of acetone and complete the acetone determination at the end of 25 minutes' use of the pumps, disconnecting the acetone apparatus. Keep up the air current and at the end of one hour and one-half detach the ammonia apparatus and complete the ammonia determination as before.

2. Another method is given by Emerson as follows: from 50 to 250 c.c. of urine are distilled until the great mass of water has passed over. To the end of the tube, which should have a good cooler, is attached a rubber tube, the end of which dips into water in the receiving flask. A little acid may be added to prevent foaming.

The distillate is poured into a graduated cylinder with a ground glass stopper, an excess (15 to 20 c.c.) of NaOH added, and then 20 c.c. of Lugol's solution which is conveniently made three times the ordinary strength. A heavy black precipitate forms which soon clears, leaving a yellow sediment of iodoform crystals.

After standing for some time, ten to fifteen minutes or more, about 40 to 50 c.c. of ether are added and the fluid shaken out until the ether contains all of the iodoform. A reading is then made of the volume of ether, 10 c.c. are removed in a graduated pipette and evaporated in the air in a weighed glass dish. This is then dried over sulphuric acid and the dish again weighed. The weight of the iodoform multiplied by 0.147 equals the weight of acetone represented in the number of cubic centimeters used. That of the whole ether extract may then be reckoned. (See also Folin-Hart method under Diacetic Acid.)

DIACETIC ACID.



Chemistry.—Diacetic acid, acetoacetic acid, or ethyldiacetic acid as it is variously termed, $C_4H_6O_3$, is formed from beta-

oxybutyric acid as shown above. It is a colorless liquid readily soluble in water, alcohol and ether, of strongly acid reaction and rapidly changed into acetone, alcohol, and carbon dioxide, when the urine begins to decompose. A characteristic of this acid is the Bordeaux-red color which it gives with ferric chloride solution, in this respect differing from acetone.

Clinical Significance.—This substance is always pathological and usually of serious import. Under-nutrition or failure of absorption is the basis of its appearance in the urine. It is rarely present without acetone. When but little diacetic acid is formed in the body, it may all be transformed into acetone, but when a larger amount is formed both appear in the urine. Diacetic acid occurs in the urine of children and young people in fevers and digestive disorders, and like acetone is then not of serious import, but when present in the urine of adults in fevers and in diabetes mellitus is of grave import.

It may be found in the urine of women in severe cases of the vomiting of pregnancy. Cases of diaceturia are sometimes noticed in cases of pure autointoxication, usually rapidly fatal in adults. It may be present in cases of gastric ulcer due to treatment by starvation or by rectal feeding.

☞ The most important cause of its presence in the urine is diabetes mellitus either in severe or advanced cases, especially in young people and in those persons who are somewhat emaciated. As a rule, the prognosis in diabetes is grave when the condition is persistent, either in those under 30 years or in older persons, though younger patients may live the longer. Appearance of it in a case of diabetes of long standing is usually a sign of rapidly approaching death. The author in an experience of thirty-two years in treating diabetes has yet to see the case which lasts many years after this substance is persistently present in the urine. It may, however, be temporarily present, when the patient is constipated, and may then not be of such serious significance.

It is claimed that diet will cause appearance of it in normal adults, and that it may also appear in mental cases with loss of weight and inanition. But it is in diabetes mellitus where presence of it is of the most interest.

Detection.—The test with ferric chloride, attributed to von Jaksch and to Gerhardt, is the common clinical test.

1. The older method of employing Gerhardt's test was as follows: to a few c.c. of urine add a strong solution of ferric chloride (perchloride of iron), drop by drop, until the precipitation of phosphates ceases. (This may be readily observed by letting the precipitate settle, after a few drops of the iron solution have been added from time to time, which it will do in about ten minutes.) Filter, and to the filtered urine add more of the iron solution. If now a Bordeaux-red color is seen, another portion of the urine is boiled and similarly treated, and if this second sample gives no reaction, suspect presence of diacetic acid. Confirm by treating a third portion of the urine with sulphuric acid, shake the mixture with ether, and draw off the ether. Test the ethereal extract with the iron solution as above, and if the Bordeaux-red be obtained, which disappears on boiling or standing for 24 to 48 hours, diacetic acid is present, especially if acetone can be detected in the distillate.

It is necessary to boil the urine, as in the second case above, since this procedure prevents the reaction with diacetic acid, but not the reaction with the urine of those who have taken various drugs (thallin, antipyrin, salicylic acid, and phenol), nor that due to fatty acids and other compounds.

If then, *after* boiling, the Bordeaux-red appears, it is due to something else besides diacetic acid.

In the author's experience the boiling test upon the urine directly, as in the second case above, is untrustworthy in cases where the urine contains a great excess of diacetic acid. In the case of an emaciated young woman, who died of diabetic coma within a few weeks of the time the author saw her, the boiled urine gave the same reaction as the unboiled.

☞ A large number of accidental constituents of urine also react with ferric chloride, as salicylic acid and salicylates, carbolic acid, coal-tar compounds (antipyrin, phenacetin, thallin), also acetic and formic acids, sodium acetate, thiocyanates and beta-oxybutyric acid. Alkapton also gives a color, but not red. Hence certain precautions should be observed in making the test.

☞ 2. **Gerhardt's Test As Used By the Author.**—As employed by the author this test is as follows: make up a 20 per cent. solution of ferric chloride, Fe_2Cl_6, iron perchloride, in water. To about an inch of urine in a test-tube add 3 drops of this solution, drop by drop, in such a way that each drop strikes the urine from a height and does not stratify. As the drops settle at the very bottom of the tube a red color is observed if diacetic acid is present. Pour some of the urine into a clean bottle and cork it tightly. Let the test-tube in which the test has been made and the corked bottle stand until the next day. Uncork the bottle and try the test again. If the color of the first test is now perceptibly lighter than that of the second, diacetic acid is present. If the two colors are just as bright, one as the other, the red is due to other substances.

The red color obtained with diacetic acid should fade on standing. In urines which have fermented diacetic acid may disappear altogether so far as this test is concerned, hence if fresh urine gives a red color with the ferric chloride and the same urine when stale fails to give it, diacetic acid was present in the fresh. Antipyretics, etc., which give a red color with ferric chloride, are not affected by the age of the urine, fermentation, etc. But all alkaline urines will give a precipitate which has a reddish tint when ferric chloride is added.

The color is, however, a deeper red than diacetic acid gives. Fresh acid urine should always be used for the test.

It is always well in testing for diacetic acid to be sure that the patient is not taking crude drugs which react with the iron solution.

3. **Lipliawsky-Arnold Test.**—This test is said to be more delicate than Gerhardt's (1:25000) and to be negative to acetone, beta-oxybutyric acid and drugs. Make up two solutions as follows: (a) potassium nitrite, 1 per cent., in distilled water. (b) para-aminoacetophenone, 1 gramme in 100 c.c. of distilled water. The solution is yellow and must be decolorized by adding hydrochloric acid, drop by drop. About 2 c.c. are usually enough and excess of acid must be avoided. Mix one part of solution (a) with two parts (b) and add an equal volume of the mixture to

that of some urine in a test-tube and mix well. Further, add a few drops of strong ammonia water and shake well. A brick-red color appears. Take about 2 c.c. of the red solution and to it add 10 to 20 c.c. of strong hydrochloric acid, 3 c.c. of chloroform and 2 to 4 drops of ferric chloride solution and mix carefuily without shaking. Diacetic acid gives a violet or blue to the chloroform. If absent, the color is yellow or light red.

Allard insists that acetone in quantity more than 1 per cent. gives a positive result with this test.

Quantitative Determination.—The methods advised by Hawk are the Folin-Hart and the Folin-Messinger-Huppert.

The Folin-Hart Method.—By this both the acetone and diacetic acid together are determined in terms of acetone. The method includes the transformation of the diacetic acid into acetone and carbon dioxide by means of heat and the subsequent removal of the acetone thus formed as well as the preformed acetone by means of an air current as first suggested by Folin. The procedure is as follows: introduce into a wide mouthed bottle 200 c.c. of water, an accurately measured excess of $\frac{N}{10}$ iodine and an excess of 40 per cent. potassium hydroxide. Prepare an aerometer cylinder containing alkaline hypoiodite solution to absorb any acetone which may be present in the air of the laboratory and between the cylinder and bottle suspend a test-tube about two inches in diameter. This large test-tube should contain 20 c.c. of the urine under examination, 10 drops of a 10 per cent. solution of phosphoric acid, 10 grammes of sodium chloride, and a little petroleum, and should be raised sufficiently high to facilitate the easy application of heat to the bottom portion. The connections on the side of the tube should be provided with bulb-tubes containing cotton. When the apparatus is arranged as described it should be connected with a Chapman pump and an air current passed through for twenty-five minutes. During this period the contents of the test-tube are heated just to the boiling point and after an interval of five minutes again heated in the same manner. By this means the diacetic acid is converted into acetone and at the end of the twenty-five minute period this acetone, as well as the preformed acetone, will have

been removed from the urine to the absorption bottle and there retained as iodoform.

The contents of the absorption bottle should now be acidified with concentrated hydrochloric acid and titrated with $\frac{N}{10}$ sodium thiosulphate and starch as in the Messinger-Huppert method.

In order to get a rough idea of how much decinormal iodine to use Hawk advises the following procedure: introduce into a test-tube 10 c.c. of the urine under examination and 1 c.c. of a solution of ferric chloride made by dissolving 100 grammes of ferric chloride in 100 c.c. of distilled water. After permitting the mixture to stand for two minutes, compare the color with that of an equal volume of the ferric chloride solution in a test-tube of similar diameter. If the two solutions be of approximately the same color intensity, 20 c.c. of the urine under examination will yield sufficient acetone to require nearly 10 c.c. of $\frac{N}{10}$ iodine solution. In case the mixture is darker in color than is the ferric chloride solution, the former should be diluted with distilled water, until it is approximately of the same intensity as the ferric chloride solution. From this data the amount of $\frac{N}{10}$ iodine solution may be roughly estimated by means of the following table:

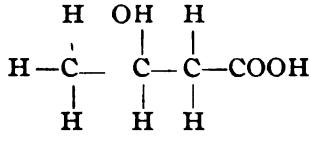
Urine cc.	Ferric Chloride.	Water.	$\frac{N}{10}$ Iodine Required cc.
10	1		10
10	1	10	20
10	1	20	35
10	1	30	50

In order to determine the diacetic acid alone proceed as follows:

Folin-Hart Method.—The apparatus is arranged as described under the Folin-Hart method for the determination of acetone and diacetic acid. The air current is started in the usual way and permitted to run 25 minutes without the application of heat to the urine under examination. Under these conditions the preformed acetone present in the solution is all removed. Attach a freshly prepared absorption bottle immediately, or introduce

fresh alkaline hypoiodite solution into the original bottle. Apply heat to the large test-tube as already described in order to convert the diacetic acid into acetone, allow the air current to continue for the usual 25 minute period, and determine the diacetic acid value in terms of acetone by the usual titration method.

β-OXYBUTYRIC ACID.



Chemistry.—This substance, $CH_3.CHO.HCH_2.COOH$, occurs in urine always in conjunction with either acetone or diacetic acid in the urine and as shown above either of the latter may be formed from it. It is not a normal constituent of urine. Isolated, it occurs as an odorless, colorless, transparent syrup which volatilizes with steam, soluble in water, alcohol, and ether, and lævo-rotatory. It may also be obtained in crystalline form. Since it rotates to the left, presence of it may be suspected when polariscopic determination of sugar gives smaller results than titration.

It is only moderately soluble in absolute alcohol and is precipitated by ether from any of its solutions. It does not react with ferric chloride. Oxidizing agents convert it into acetone and diacetic acid, of which it is the mother-substance.

☞ **Clinical Significance.**—It occurs in urine as an abnormal constituent and in largest quantity is found in severe cases of diabetes mellitus. It has been thought to be the cause of the acid intoxication which usually precedes and accompanies coma, but in late years its clinical significance has been questioned. It acompanies acetone and diacetic acid, but is not always present with these bodies.

Herter claims that persistent excretion of more than 25 grammes in 24 hours is a sign of impending coma. In smaller quantity it has been found in the urine of scurvy, scarlet fever, measles, insanity, starvation, cancer coma, and in the case of persons living on an exclusive meat and fat dietary. In the latter

case about 9 grammes in the 24 hours have been found. In severe cases of diabetes mellitus from 50 to 100 grammes have been found and in rare cases more than two hundred. Occasionally, the acid is found in the urine of diabetes mellitus for a long period of time without development of coma, but, according to Croftan, rapid decrease of acetone coupled with a corresponding rapid increase of beta-oxbutyric acid and of diacetic acid is a particularly bad omen. Stern insists that its significance is much over-rated.

Pathology.—The origin of this acid in the body, as well as that of acetone and diacetic acid, is, according to some authorities, the breaking down of fatty tissues, due to deranged metabolic conditions. When large amounts of the three acetone bodies are found in the urine, the condition is termed *acidosis*. The deranged metabolic condition leading to acidosis is thought to be cellular asphyxia, *i. e.*, interference with intra-cellular oxidation.

Beta-oxybutyric acid is a homologue of lactic acid, and appears to be formed, according to other authorities, from diseased muscle tissue, while lactic acid is formed from healthy tissue. More plausible, says C. E. Simon, is the supposed origin from the carbohydrates, especially from the hexoses. During catalysis of these substances lactic acid is formed, which is readily decomposed into formic acid and acetic aldehyde. Condensation of two molecules of the latter yields the aldehyde of beta-oxybutyric acid, which in turn on oxidation yields the acid. The whole subject of the mode of formation of this acid in the body is still in doubt.

Detection.—The only test which can be called clinical is that of Black.

1. Black's reaction: 10 c.c. of urine are slowly concentrated by gentle heat to about 3 cubic centimeters. A few drops of concentrated hydrochloric acid are added, and enough plaster-of-Paris to make a thick paste. Let the mixture stand in the cold until it begins to set, then break up by stirring until a porous meal is obtained. Treat twice with ether, stirring and mixing well, and any beta-oxybutyric acid present will be extracted by the ether. Let the ether extract evaporate spontaneously, dis-

solve the residue in water and neutralize with barium carbonate. Add to from 5 to 10 c.c. of the neutral fluid 2 or 3 drops of commercial acid hydrogen "peroxide," mix well by shaking and add a few drops of Black's reagent (5 grammes of ferric chloride and 0.4 gramme of ferrous chloride in 100 c.c. of water). Let the tube stand and note the gradual development of a rose color which increases until a certain maximum is obtained, after which it gradually fades. In order to obtain the best results Hawk stipulates that the aqueous solution must be cold and approximately neutral, and that a large excess of peroxide and of Black's reagent must be carefully avoided. The color will be destroyed or will be only transitory if too much of the oxidizing agents are added. It is better to add a few drops of the reagent, wait a few minutes, and then to add a few drops more until the color undergoes no further increase in intensity. It is claimed that this test shows 1 in 10,000.

The principle of the test is the oxidation of beta-oxybutyric acid into diacetic acid, which gives a red color with ferric chloride. It can not be applied directly to the urine on account of interfering bodies, viz., urinary pigments, sugar, and diacetic acid.

2. **Külz's Test.**—When beta-oxybutyric acid is boiled with dilute mineral acids alpha-crotonic acid is formed, hence the following test: ferment the urine to remove sugar and evaporate it to a syrup, add an equal volume of concentrated sulphuric acid and distil while still hot. The distillate contains alpha-crotonic acid. Let it cool slowly and note the formation of crystals of alpha-crotonic acid which melt at 72° C. (161.6° F.) and are soluble in ether. If no crystals are obtained, shake up the distillate with ether, pipette off the ethereal extract, let it evaporate, wash with water, and crystals of alpha-crotonic acid may be obtained.

3. **Polariscopic Test.**—After fermenting the urine examine with the polariscope after clarifying by addition of solution of neutral lead acetate and filtering. Rotation to the left in the absence of the conjugate glycuronates (determined by the naphthoresorcin test, etc.) indicates presence of beta-oxybutyric acid.

Clinical Quantitative Determination.—The easiest method is the polarization of the fermented urine, the lævo-rotation remaining after the urine has been clarified with basic lead acetate and ammonia. A good clinical method, according to Emerson, is the difference between polarization and titration, both being accurately done; or the results of fermentation with Lohnstein's apparatus may be compared with polariscopic determination. Comparative tests made from day to day by these methods will show whether the amount of beta-oxybutyric acid is increasing or decreasing.

Other Quantitative Determinations.—The various methods are as follows:

1. **Method of Black.**—This is practically the same as the qualitative test and is performed as follows: render 50 c.c. of the urine faintly alkaline with sodium carbonate and evaporate to one-third its original volume. Then concentrate on the water-bath to about 10 c.c., acidify with a few drops of concentrated hydrochloric acid until a distinctly red color is imparted to blue litmus paper and add plaster-of-Paris to form a thick paste. Let stand until it begins to set, then break up with a thick glass rod having a blunt end and reduce to the consistency of a fairly dry coarse meal adding more plaster if necessary. Transfer the meal to a Soxhlet apparatus and extract with ether for two hours, after which let ether extract evaporate. Dissolve the residue in water, filter, if necessary, adding a little bone black until a clear solution is obtained, and make up the filtrate to a known volume, 25 c.c. or less, with water.

In the solutions thus obtained the amount of beta-oxybutyric acid is determined by polariscopic examination.

The patient should avoid taking benzosol when any polariscopic determination is to be made, for this substance is lævo-rotatory.

2. **Shaffer's Method.**—This method is given first place by Hawk and is performed as follows: according to the color obtained with ferric chloride solution applied directly to the urine, introduce from 25 c.c. to 250 c.c. of urine into a 500 c.c. volumetric flask (if the color with ferric chloride is strong but 25 to

50 c.c. of urine will be needed). Add excess of basic lead acetate and 10 c.c. of strongest ammonia water. Fill up with distilled water to the 500 c.c. mark, shake vigorously, and filter.

Transfer 200 c.c. of the filtrate to an 800 c.c. Kjeldahl distilling flask, add 300-400 c.c. of water, 15 c.c. of concentrated sulphuric acid and a little talcum and distil until 200-250 c.c. of distillate have been obtained. The distilling flask should have a dropping-tube by means of which water may be introduced in order to prevent the contents of the flask from becoming less than 400 c.c. in volume. A good condenser is also absolutely necessary, but ice need not be employed.

The distillate obtained as above contains acetone and volatile fatty acids, including formic, which latter must be removed. Hence add to the distillate 5 c.c. of a 10 per cent. potassium hydroxide solution and redistil. This second distillate is now titrated with standard iodine and thiosulphate solution. Then the residue from the first distillation is again distilled and during the process there is added by means of the dropping-tube from 400 to 600 c.c. of a 0.1 to 0.5 per cent. solution of potassium dichromate; as a rule, addition of 0.5 gramme in all of the dichromate is sufficient, unless the urine is loaded with sugar, or unless a large volume of urine is used, when 2 to 3 grammes in all of dichromate must be added. In adding the latter, care must be taken not to add faster than the distillate collects, except in cases where the boiling fluid assumes a pure green color, which shows that the dichromate is being used up more rapidly. After about 500 c.c. of distillate have been collected, add to it 20 c.c. of a 3 per cent. hydrogen dioxide solution and a few c.c. of a potassium hydroxide solution and redistil. When 300 c.c. are collected, titrate this also with iodine and thiosulphate. Hawk advises use of solutions a little stronger than decinormal, viz., 103.4 $\frac{N}{10}$. Each c.c. of this stronger iodine solution is equivalent to 1 milligram of acetone, or 1.794 milligram of beta-oxybutyric acid. The solution should be restandardized from time to time with decinormal potassium bi-iodate.

Titration with iodine and thiosulphate has been described under Acetone (Messinger-Huppert method), which see.

3. **Darmstaedter's Method.**—The method is based on the decomposition of the β-oxybutyric acid with the formation of α-crotonic acid and the estimation of the latter. One hundred c.c. of urine are rendered feebly alkaline with sodium carbonate and evaporated on the water-bath almost to dryness. With the aid of 150-200 c.c. of sulphuric acid (50-55 per cent.) the residue is transferred to a liter flask, which is closed with a doubly perforated stopper. Through the one aperture a drip-tube passes, while a bent glass tube passes through the other to a condenser. Heat is applied, at first mildly, so as to avoid foaming; then vigorously. Water is allowed to enter through the drip-tube as fast as the distillate passes over. The distillation is interrupted when from 300 to 350 c.c. have been obtained, which usually takes from two to two and one-half hours The distillate is extracted several times with ether in a separatory funnel. The ether is distilled off, then the residue heated for a few minutes on a sand-bath to 160° C. in order to drive off any fatty acids that may be present, and then dissolved on cooling with 50 c.c. of water. The solution is filtered and the filter washed off with a little water. The aqueous solution of crotonic acid is now titrated with a decinormal sodium hydrate solution, using phenolphthalein as an indicator. One c.c. of the soda solution corresponds to 0.0086 grammes of crotonic acid. The corresponding amount of β-oxybutyric acid is obtained by multiplying by 1.21. Sugar does not interfere with the process. To compute the quantity of β-oxybutyric acid in grammes multiply the number of c.c. of decinormal sodium hydroxide used by 0.01041.

The extraction of the distillate with ether in the separatory funnel should be faithfully carried out; three times is not enough, according to Shaffer.

4. **The Strassburg Clinic Method.**—About 400 c.c. of urine are evaporated to about 100 c.c. This is roughly saturated with ammonium sulphate, and evaporated to 60 c.c. The ammonium sulphate removes the albumin and some of the coloring matter of the urine; the concentrated solution of salts is of advantage since oxybutyric acid is much more soluble in water than in ether.

When perfectly cool it is filtered. It is then made strongly acid with sulphuric acid, placed in a liter flask nearly filled with ether and shaken violently for about ten to fifteen minutes. After standing until the urine is settled and the ether solution of the oxybutyric acid is nearly clear, the ether is poured into a separatory funnel, and in half an hour or less 20 c.c. of distilled water are added, and the fluid shaken violently ten minutes. This will remove some of the sulphuric acid as well. The water is allowed to separate for thirty minutes. This wash water is saved for the following ether extractions. The ethereal extract is preserved in a large flask. The original urine is again extracted in the same way and this ether extract washed with the same wash water and added to the above. This process is repeated at least five times, the ether extracts after washing with this same wash water being combined. This wash water after each ether extract will contain less of the oxybutyric acid, the most going to the ether, and may be finally thrown away. The united ether extracts are then distilled at 60° to 70° C., until all of the ether has passed over.

In the residue is the oxybutyric acid. This is transferred to a porcelain dish, the distilling flask washed with ether, and the residue of this, when evaporated, added to the above. A little distilled water is then added, which causes a slight cloudiness, due to hippuric acid. The solution is allowed to stand at 24° C. It is then filtered, using a knife point of salicylic acid to clarify it, until clear. It is then washed with distilled water, and the final volume brought to 25 c.c. The angle of polarization is then determined, using a 1- or 2-decimeter tube. In a 1-decimeter tube (α) $D = -24.16°$. The rotation determined by this will give the percentage of acid in the original amount of urine.

Ten c.c. of the solution used in polarization may be titrated with fifth-normal sodium hydroxide, using phenolphthalein as indicator. The number of c.c. necessary multiplied by 0.104 equals the number of grammes of oxybutyric acid in 10 c.c.

Bergell's Method.—Render 100-300 c.c. of sugar-free urine, slightly alkaline with sodium carbonate, evaporate the alkaline

urine to a sirup on the water-bath, cool the sirup, rub it up with sirupy phosphoric acid (being careful to keep the mixture cool), 20-30 grammes of finely pulverized anhydrous cupric sulphate and 20-25 grammes of fine sand. Mix the mass thoroughly, place it in a paper extraction thimble and extract the dry mixture with ether in a Soxhlet apparatus. Evaporate the ether, dissolve the residue in about 25 c.c. of water, decolorize the fluid with animal charcoal, if necessary, and determine the content of beta-oxybutyric acid by a polarization test.

Boekelman and Bouma's Method.—Place 25 c.c. of urine in a flask, add 25 c.c. of 12 per cent. sodium hydroxide and 25 c.c. of benzoyl chloride, stopper the flask, and shake it vigorously for three minutes *under cold water.*

Remove the clear fluid by means of a pipette, filter it and subject it to a polarization test. Through the action of the benzoyl chloride all the lævo-rotatory substances as carbohydrates, albumin, etc., except beta-oxybutyric acid will have been removed, and the lævo-rotation now exhibited by the urine will be due entirely to that acid.

CHAPTER XXV.

URINARY SEDIMENTS.

Sediments are conveniently classified for clinical purposes into chemical and microscopical, *i. e.*, those which can be recognized by chemical tests alone, and those for identification of which the microscope is absolutely necessary.

Physical and Chemical Recognition of the Sediment.—The sediments which can be recognized by inspection and by physical and chemical tests are the following: urates, uric acid, phosphates, carbonates, oxalate, pus, blood and mucus.

☞ (I.) By *inspection* we differentiate them as follows: take the reaction of the urine with litmus paper first. (1) A mealy sediment of clay color, light yellow, yellow, reddish or pinkish in acid urine is composed of urates, viz.: the amorphous urates of sodium and potassium. In young children this sediment is somewhat milky in appearance. (2) A dense, heavy sediment of brick-dust appearance in acid urine, tending to settle at the edges and corners of the bottom of the glass, with a lighter clay-colored cloud above it, is *uric acid, i. e.*, crystals of free uric acid. (3) A copious light-colored flaky sediment, easily disturbed, in feebly acid, neutral or alkaline urine, is likely to be composed of *phosphates* and *carbonates*, amorphous phosphates in odorless urine, but crystalline (triple) in ammoniacal urine. In the urine of women such a sediment may also be composed of *mucus* and *epithelium*. (See microscopic examination.) The sediment of phosphates, when shaken up with the urine, renders it cloudy, but not densely so. (4) A light-colored, slight, easily moving sediment is likely to be composed of oxalate, *i. e.*, *calcium oxalate* crystals. (See further on.) (5) A heavy, rapidly settling, whitish flocculent sediment in acid urine suggests presence of *pus*. Before settling, pus suspended in acid urine appears as comparatively large, dirty white flocks, larger than phosphates. (6) A heavy, greenish, slimy sediment in alkaline urine

sticking to the glass is *pus,* occurring in clots and gelatinous masses, so rendered by the action of the alkali in the urine. The sediment described above (5) will become stringy and viscid when the urine changes from acid to alkaline. In pouring the urine from one glass to another the pus tends to pass over all together in a lump or to string out in a long, viscid string. (7) A whitish sediment in the urine of women with a hazy appearance throughout the supernatant urine is due to *epithelium, mucus* and *micro-organisms,* as in leukeorrhea. (8) Urine, cloudy when voided and which remains cloudy throughout its entire bulk, no matter how long it stands, contains *bacteria,* as in cystitis. (9) Urine containing sugar deposits an abundant light-colored sediment as it grows stale, due to excess of acidity and formation of *penicillium glaucum,* the so-called "mother" in acid liquids. More frequently the mass is suspended in the urine while red sand (red pepper grains of uric acid) are seen in the sediment. (10) If the sediment is reddish in color it is composed of *blood,* but urates and uric acid may have a reddish tint also. As a rule, the latter, however, are more pink, or like brick-dust in color, while blood is brighter red or much darker. Urine containing blood may also have clots and on standing a beef-tea or smoky hue is apparent in the whole volume of urine, whereas urine containing a sediment of urates or uric acid is likely to be normal in appearance above the sediment. With reference to the freshly voided urine we find **urine cloudy when voided** to contain any sediment except urates and uric acid. Urine clear when voided, but becoming cloudy on cooling, owes its sediment to the presence of urates and perhaps, also, uric acid. In only one case has the author noticed urates in the sediment of warm acid urine. (11) Urine apparently clear when voided, but which, on standing, soon shows a slight cloud floating in about the middle of the mass of liquid, contains a *mucous cloud,* or nubecula, which consists of mucus, epithelium and epithelial debris from the genito-urinary tract. Such a cloud is common in the freshly voided urine of women and in catarrhal conditions of the lower urinary tract in either sex. (Addition of acetic acid causes the mass to sink.) (12) Urine containing small,

shreddy, cottony masses owes its peculiar appearance to presence of "gonorrheal threads," so-called, which may persist for years after cure of the infection. (13) Urine containing slimy masses in relatively small amount in the sediment and which are not sticky nor greenish, but transparent, is likely to show evidences of the presence of prostatic or seminal fluid.

☞ (II.) By *physical* or *chemical tests* we recognize the sediment as follows: take the reaction of the urine as soon as received. If acid, test the sediment for urates, uric acid, calcium oxalate, pus and blood. If feebly acid, neutral or alkaline, test for carbonates, phosphates, oxalate, pus and blood. For collection of the sediment the centrifuge is most desirable. (See microscopical examination.) In case no centrifuge is at hand, let a sample of the well mixed urine settle for several hours in a tapering glass vessel holding about six ounces. To obtain the sediment for testing use a pipette or ordinary glass tube of ordinary caliber, long enough to dip well down to the bottom of the tapering glass, but not longer. Close the upper orifice of the tube tightly with the finger, dip the lower end into the sediment and remove the finger: urine rich in sediment runs up into the tube; again close the upper orifice of the tube with the forefinger and remove the tube from the glass. The urine and sediment in the tube do not flow out as long as the finger is tightly pressed over the upper orifice. Insert the lower orifice into a test-tube, remove the finger, and the sediment will now flow out into the test-tube, where it may be tested in various ways, as provided further on. In this book, under the heading "Chemical Tests for Sediments," it is always assumed that the sediment to be tested has been removed to a test-tube in this way, unless the centrifugal machine is used. When several chemical tests are to be tried, it is best to use several samples of the sediment in different test-tubes.

☞ **Acid Urine.**—1. Hold the tube containing sediment in water heated to 60° C. (140° to 150° F.). If it clears wholly or partly, *urates* compose the sediment.

2. If it does not clear with heat, is of a brownish or yellowish color, and consists of small grains looking like "red pepper grains," add a few drops of liquor potassæ to the sediment and shake; if it dissolves, *uric acid* is present. The crystals of uric

acid can be collected on a filter, washed off into a dish and tested with the murexide test. (See Chapter VII.)

3. If the sediment is small in amount and colorless, divide into three portions, add acetic acid to one and hydrochloric acid to the other, and shake thoroughly. If the second is dissolved and the first is not, *calcium oxalate* is probably present. Confirm by adding a few drops of liquor potassæ to third portion, and notice insolubility. For this test it is important to obtain the sediment free from urine, hence the centrifuge is virtually a necessity. However, the physician should not rely on chemical tests alone for the detection of oxalate, since it occurs in such small amount, usually, as to render detection difficult except by the microscope.

4. If the sediment is whitish, and especially if it is dense and creamy white, add a few drops of liquor potassæ to it. If it becomes greenish and gluey, perhaps forming viscid strings when poured from the tube, *pus* is present. The urine itself, if pus is present, will always respond to the albumin test, showing from traces to one-half on Esbach tube. The chemical test for pus as above is not always satisfactory when but little pus is present, and the physician is advised not to rely upon it in case it is negative; but a gelatinous, glairy lump found after addition of alkali to the sediment always indicates pus. (Donné's Test.)

5. If the sediment is reddish in tint and does not respond to tests for urates nor appear as uric acid crystals, test for *blood* as follows: mix equal parts of freshly made tincture of guaiac and old spirit of turpentine, shake well and cause a like amount of urine containing the sediment to trickle down the side of the tube into the guaiac-turpentine mixture. A dense, yellowish precipitate (guaiac) is seen in all urines, but, if blood be present at the juncture of the yellow precipitate and fluid above it, there is seen a *blue color,* slowly appearing. Use freshly voided urine in making this test. The urine itself will always respond to the albumin test, showing from traces up to the second mark or even higher on Esbach tube, according to amount of blood. In case the guaiac test fail, use the tests already described in Chapter XX. on Hematuria.

☞ **Feebly Acid, Neutral or Alkaline Urine.**—Test a *whitish* sediment for phosphates and carbonates by adding acetic acid and shaking well. If phosphates and carbonates alone are present, the sediment is wholly dissolved. If the sediment is only partly dissolved, seen by comparison with some of the original sediment in another tube, to which nothing has been added, phosphates are present together with other constituents, probably micro-organisms, mucus, epithelia, and perhaps pus.

If the addition of acetic acid to the sediment causes noticeable effervescence, carbonates are present along with the phosphates. If the urine has an odor of ammonia, triple phosphate and ammonium urate are likely to be present in the sediment.

If the sediment is not noticeably affected by acetic acid and is glairy or sticky, *pus* is probably present, altered by the action of the alkali. Pouring the urine from one glass to another will cause the sediment to pass over in one lump or in a few lumps, if pus is present in alkaline urine.

If the sediment is not noticeably affected by acetic acid and is light, easily disturbed and flocculent, it may be composed of *oxalate*, which may also be present in alkaline urine.

A reddish sediment in alkaline urine is likely to be *blood*, since amorphous urates and uric acid do not occur in the sediment of fresh alkaline urine.

☞ **Urine of Any Reaction.**—A whitish sediment in the urine of women is likely to be composed of *mucus, epithelium, leukocytes* and *bacteria* from urinary and vaginal mucus, and occurs in urine of any reaction. Such a sediment is not dissolved by warming nor by acids. The microscope is necessary for complete identification, but excess of mucus in urine not containing pus may be inferred from the slowness with which it filters.

Urine of any reaction and of a hazy appearance, which does not settle even on standing for days, owes its haziness to the presence of bacteria. These require for sedimenting a high speed in the centrifugal machine,—2,000 revolutions per minute or more. They are common in genito-urinary troubles or in affections of the lower urinary tract, leucorrhea, etc., and in stale urine especially. Presence of them should always suggest tardy delivery of the urine to the physician.

CHAPTER XXVI.

THE MICROSCOPICAL EXAMINATION OF URINE. UNORGANIZED SEDIMENTS.

The microscope and accessories.

Collection of the sediment: the centrifuge and accessories.

Preparation of the sediment for examination: collection of the urine; sedimentation; use of the pipette; examination with the low power and with the high; focussing.

Staining the sediment: Lugol's solution; Saxe's, Emerson's, Cohn's and Erdman's methods.

Preservation and mounting of sediments: how to preserve crystals; how to preserve casts, corpuscles, etc.; Mueller's, Bohland's Farrand's and Heitzmann's methods; use of salt and corrosive sublimate.

Mounting the sediment: methods of Delépine, Mueller, Cohn, and Farrant; how to "ring" slides.

Identification of sediment: combination of eye-piece and objective; suggestions from the color of objects; things easily seen with the low power; things requiring a high power for identification; crystalline objects; amorphous objects; crystalline sediments of common occurrence; crystals commonly found in acid urine; in neutral urine, etc.

Crystalline sediments of less common occurrence: crystals having color and colorless crystals; large crystals and small.

Sediments of uric acid described in detail; clinical significance.

Sediments of crystalline urates described in detail; clinical significance.

Amorphous sediments: urates and phosphates; comparison with bacteria.

Sediments of amorphous urates described in detail; tests and significance.

Sediments of calcium oxalate described in detail; microscopical appearances; different forms; physiology, pathology and significance; relation to albuminuria, urethritis, etc.; claims of Swinburne and Lewis.

Sediments of the various phosphates.

Sediments of the ammonium-magnesium phosphate:—occurrence, chemical constitution, color, appearance, and form; microscopical appearance; physiology and pathology.

Sediments of stellar phosphate (acid calcium phosphate); properties, appearance, physiology, pathology, etc.

Sediments of earthy phosphates: properties, appearance, tests, physiology, pathology, etc.

Amorphous carbonates.

Crystalline sediments of infrequent occurrence: calcium carbonate, calcium sulphate, magnesium phosphate, cystine, etc.

Cystine sediments: properties, tests, microscopical recognition, reactions, significance, etc.

Creatinine, cholesterine, hippuric acid, leucine, tyrosine, hematoidin, indigo, xanthine, melanin, hetero-albumose, hemoglobin.

Fat in the sediment: chyluria, lipuria; significance.

☞ For urinary work the Bausch & Lomb microscopes (Fig. 33) of reasonable price are sufficient. The Abbé condenser is removed in order to obtain a strong diffused light. *The author greatly prefers a 3/4-inch eye-piece in combination with a 1/2-inch objective and a 1/5.* When searching for hyaline tube-casts, shade the mirror with the left forefinger. By the combination above described so much working distance is obtained that the objective is not easily in contact with the urine. In general whatever microscope is used, much time and annoyance will be saved by a combination of eye-piece and objective which allows focussing at some distance above the surface of the urine upon the slide. Any microscope in which the focussing brings the object glass close to the urine is a nuisance in urinary work and should not be used. The trouble may usually be remedied by changing the eye-pieces, etc. Do not use the usual combination of 2/3 and 1/6 objectives; the one is too low, the other too high. In addition to the microscope, glass slides and pipettes are necessary. For ordinary urinary work keep the *slides* in a wide mouthed glass,—an ordinary tumbler will do,—containing a frequently renewed solution of an antiseptic soap. Before using wash and wipe dry with soft cloth. If the slide is greasy (shown by the inability of the urine to spread evenly over it), soap it, wash, and dry. If it becomes scratched, throw it away.

☞ **Pipettes** are practically small glass tubes drawn to a point at one end and smoothed or ground at the other. The writer prefers to use merely a glass tube of about 1/8-inch diameter,

not drawn to a point at the lower end. The tube should be just long enough to dip down to the bottom of the centrifugal tube used. In such a tube the entire sediment can usually be drawn up from 10 c.c. of urine, so that the constituents of different layers of the sediment can all be removed at one dip.

Pipettes should be immersed after use in a solution of anti-



FIG. 33.—Bausch & Lamb Microscope.

septic soap and rinsed out before use in running water. Care should always be taken not to dip the same pipette without cleansing into one urine sediment and then into another.

Collection of the Sediment.—If the physician does not possess a centrifuge, the urine should be allowed to stand several hours

in a tapering glass vessel called a sediment glass (Fig. 34), covered over with any suitable material, like glass, to keep out the dust. Boric acid in proportion five grains to every four ounces should be added as a preservative and well shaken up before the urine is allowed to settle. By use of a ground glass cover and the sediment-glass urine may be kept in a good condition for many hours, if necessary, in order that the sediment may collect.

☞ But the best way to collect a sediment is to use the *centrifuge* in combination with freshly voided urine. Centrifugal machines (Figs. 35, 36, and 37) are operated by hand, water, or electricity. The cheapest are the hand machines, but these are tiresome, hence those in which water or electricity are used are preferable. The best centrifuge is the Purdy electric



FIG. 34.—Sediment Glass.

model with arms of such length that the tips of the tubes describe a circle of standard diameter, namely, 13½ inches, hence suited also for the quantitative work already described. For microscopical work, however, it is not necessary that the circle described should be of the standard diameter.

☞ The author has devised *special centrifugal tubes* for microscopical work, made for him by E. H. Sargent & Co., holding only 10 c.c. of urine (instead of 15 c.c.) and drawn out to a long tapering extremity instead of a short blunt one. Each of these tubes filled with 10 c.c. of urine weighs only 18 grammes. Being drawn out to a fine extremity the entire sediment of 10 c.c. of urine is condensed into such small bulk that it can be readily withdrawn with a pipette or glass tube of small caliber and placed entire upon a slide.

In this way count of tube-casts per 10 c.c. of urine can be made from time to time and the relative number of corpuscles, crystals, etc., observed. The special tubes should always be used in preference to larger and heavier ones ordinarily sold. Smaller aluminium guards may be used with these tubes; being lighter, a greater relative speed is possible. The physician should notice that the speed of a centrifuge can not be regulated at all times of the day and night by its rheostat, as claimed, if the ordinary light current is used, since during the day time this current may produce less relative speed than after dark.



FIG. 35.—The Purdy Electric Centrifuge.

The Purdy centrifuge can be operated either by battery currents or by the street, and as high a speed as 10,000 revolutions per minute with small bacterial tubes is claimed for it. The machine is made with both two arms and four arms, the latter being useful in cases where much work is to be done, the former sufficient for the general practitioner. The machine should be screwed down on a heavy table or specially constructed shelf capable of supporting it without jarring while running. On such support the Purdy machine runs almost noiselessly. It is

well to surround the machine with a sheet-iron circular guard made to order for the purpose of protecting the physician from flying glass, etc., in case anything breaks. The Purdy machine, although somewhat expensive, lasts for years.

☞ The sediment is collected and prepared for examination as follows: obtain, if possible, three samples of urine, viz., the day, the night, and a freshly voided sample. Examine the freshly



FIG. 36.—Hand Centrifuge.

voided first. Shake it up gently (not violently), and fill each of the two centrifugal tubes with it. Cork the latter with cork obtained by cutting in halves an ordinary small cork adapted to the size of the mouth of the tube and set in the aluminium guards. Move the rheostat (at the bottom of the machine) until a speed of 1,000 to 1,500 revolutions per minute is obtained, and subject the urine to such speed for five minutes. When removed from the machine the sediment will be found at the bottom of the tapering extremity and the supernatant urine may be poured off from this sediment without disturbing it.

☞ Place the forefinger lightly over the top of a pipette and introduce the pipette into the tube so that its mouth touches the bottom. Remove the finger from the top of the pipette, which, if its mouth is wide enough, will allow all the sediment to rise in it. Then replace the finger over the top of the pipette and remove the inclosed sediment to a clean glass slide. Allow the sediment to flow evenly over the slide and place the latter in the field of the microscope. The latter should have a nose-piece containing both objectives. Use first the 1/2-inch objective, which with the ¾-inch eye-piece, flat mirror, and no condenser,



FIG. 36*a*.—Water Power Centrifuge.

should, when focussed, show a large, well-lighted field in which the *general character* of the sediment can be observed. Having observed the general character of the sediment with the lower power, then change to the 1/5 or 1/6-inch objective and study the *special character* of the objects.

☞ Casts should be sought for with the 1/2-inch, shading the mirror with the forefinger. Corpuscles (blood, pus, etc.) require the 1/5-inch for identification, as do also fat and fat in casts, small epithelia, spermatozoa, and small crystals. While casts may be found by the ½-inch, or any low power used, the

special character of them must be studied with the high (1/5 or 1/6-inch).

☞ Use of the fine adjustment of the microscope is necessary in connection with the high power (1/5 or 1/6) in order to see casts in different planes. Beginners are frequently puzzled by the fact that there are *two planes* in each of which the microscope focusses: first, that of the glass showing only the cracks, roughnesses, etc., in the glass, and second, that of the urine. The focus necessary is one in which *moving objects* are seen, *i. e.*, that of the urine containing objects floating in it, ***hence keep changing the focus until moving objects are found and then move the slide until a number of these objects appear.***

A north light during the daytime is preferable for microscopic purposes and after dark a small lamp placed in front of the microscope. Artificial light should not be so strong as to affect the eyesight of the operator.

STAINING THE SEDIMENT.

The various formulas for staining are in the main unsatisfactory in urinary work. The best general stain for casts is *Lugol's solution* (iodine in aqueous iodide of potassium), a drop or two added to the sediment of 10 c.c. of urine obtained by centrifuging. Casts are stained deep yellow to brown, according to amount of stain present. This stain shows crenated red blood corpuscles well, and may serve to find them in a mass of leukocytes. It also shows non-crenated red cells as round, smooth, coin-shaped objects.

To demonstrate nuclei of epithelia and leukocytes Saxe washes the sediment repeatedly with distilled water, lets dry upon a slide, covers with equal parts of absolute alcohol and ether, lets dry again, stains with saturated alcoholic eosin one part, water four parts, for 30 seconds, washes with distilled water, adds 2 per cent. aqueous methylene-blue, stains 2 to 5 minutes according to thickness of film, and washes well in distilled water. The nuclei appear blue or purple, cell bodies a faint pink and bacteria deep blue or purple.

According to Emerson the following method may be pursued

for staining casts: the sediment should be washed by one or two sedimentations with 0.6 per cent. NaCl solution. After two decantations the tube is filled up with water containing 1 per cent. methylene blue and just a little alcohol, sedimented in the centrifuge for a short time, and immediately decanted. Cohn stains fat and cell-nuclei as follows: wash the sediment thoroughly by centrifugalizing once or twice in 0.6 per cent. sodium chloride solution, let dry on a cover-glass and immerse the glass for 10 minutes in 10 per cent. formalin. Wash rapidly but gently in water and immerse for 10 minutes in a concentrated Sudan III. solution in 70 per cent. alcohol. Wash with 70 per cent. alcohol for one or two minutes and stain quickly in Ehrlich's hematoxylin.

PRESERVATION AND MOUNTING OF SEDIMENTS.

Urinary sediments for demonstration by instructors may be bottled and preserved as follows:

Phosphatic Crystals.—Wash the centrifuged sediment several times by decantation with a little weak ammonia water (1 per cent.) which is left in the last washing and the whole poured into a small clean bottle, tightly stoppered with a glass stopper. Since these crystals do not keep many months, it is well to mount them soon after washing.

Urate Crystals.—Wash several times by decantation with a small volume of 33 per cent. alcohol and treat as above.

Uric Acid, Hippuric Acid, Oxalate, Cystine, Cholesterine.—Wash as above with 1 per cent. acetic acid. A. S. Delépine preserves the crystalline sediments dry by washing with distilled water, centrifuging and decanting three or four times till all soluble parts of the urine have been removed, drying on the water-bath below 40° C. (104° F.) and placing in a small clean, tightly stoppered glass bottle.

Organized Sediments.—Epithelia, casts, blood, pus, fibrin, fat, connective tissue, etc., may be treated in various ways: Ogden advises centrifuging and washing by decantation twice with 4 per cent. boric acid, then three times with aqueous solution of potassium acetate of specific gravity 1030, containing 1 per cent. formalin, leaving the sediment in the last washing and placing in

a tightly stoppered bottle. Blood corpuscles naturally change (become smaller and lose their color), but otherwise the sediment suffers little change in months or even years.

For preservation of casts and other organized deposits *Mueller's* fluid may be used, made by dissolving potassium dichromate 2 parts and sodium sulphate 1 part in 100 parts of water. The urine is first well centrifuged, the supernatant fluid decanted off, and Mueller's fluid diluted with equal parts of water stirred into the sediment. The sediment is allowed to settle, the supernatant fluid is poured off and diluted, Mueller's fluid again added. This treatment is repeated three times in all and finally the undiluted Mueller's fluid added in which the sediment is allowed to remain for two weeks. The fluid is then decanted and the sediment thoroughly washed with 50 per cent. alcohol. The alcohol is decanted off and the sediment placed in a solution composed of ten parts each alcohol, glycerin, and water, and one part carbolic acid, the whole in a tightly stoppered bottle. It is claimed that by this method the sediment may be preserved for years.

Bohland advises to wash with salt solution, decant and add Mueller's fluid, keep the sediment in this for two weeks, changing the fluid several times, decant and wash the sediment with absolute alcohol until colorless.

Farrant's fluid is sometimes used for casts: equal parts of water, glycerin, and saturated aqueous solution of arsenic acid (saturated by weeks of standing), are added to one-half volume of gum-arabic solution and the whole let stand for three weeks until all is dissolved, when it is filtered, if necessary. This solution is chiefly used for mounting purposes.

The method of the late Charles Heitzmann was to centrifuge the urine, decant, and add to the thickest sediment obtainable a few drops of strong chromic acid solution. Let stand a week, then gradually add a little pure glycerin, day after day, for three or four days. Let stand for a few days more until all water has evaporated, then mount without any addition, clean, and surround with asphalt. The exact amounts of chromic acid and glycerin must be learned by practice.

Casts may be preserved and stained by washing once or twice

with 0.6 sodium chloride solution, and lastly, with either a 1 per cent. osmic acid solution, a 1 to 10 formalin solution, or a 5 per cent. mercuric chloride solution for five minutes. If mercuric chloride is used, wash with water and keep in 1 per cent. formalin. If the 1 to 10 per cent. formalin is used, wash well once with water to prevent crystalline formations.

For staining fat and cell nuclei Cohn advises that the specimen well washed by centrifugalization in normal salt solution be air dried on the cover glass and hardened by immersing the glass in 10 per cent. formalin. Wash rapidly, but gently, in water and immerse for 10 minutes in a concentrated Sudan III solution in 70 per cent. alcohol. Wash in 70 per cent. alcohol for one or two minutes and stain quickly in Ehrlich's hematoxylin.

Erdman's Method of Staining Granular Casts and Other Tube Products.—Sediment the fresh urine in a centrifuge; pour off the supernatant urine, and fill the tube with 0.4 per cent. sodium chloride solution. Gently shake the deposit, centrifuge, pour off the solution, and repeat the washing, this time centrifuging till the sediment forms a dense clump at the bottom of the tube. Slowly pour off the solution, invert the tube and allow it to drain for a few moments; the sediment will remain in the point of the tube. With a long pipette transfer a small drop of sediment to a cover-glass, gently spread with a fine platinum wire loop, and dry in air. The spread must be thin. Treat for three minutes with a 5 per cent. aqueous solution of mercuric chloride. Wash thoroughly in water. Stain for five minutes with a fresh mixture of equal parts of the following solutions: methylene blue, 0.3 per cent. aqueous solution; fuchsin, 0.02 per cent. aqueous solution; wash in water, dry, and mount in balsam. To demonstrate fat, stain the fixed spread for five minutes with a solution of Sudan III in 70 per cent. alcohol. Wash in water, and apply the double stain as before. Mount in glycerin or glycerin jelly. Mucin and chromatin stain violet to indigo, granular casts and protoplasm stain from pink to dark red, waxy casts stain bright red and fat globules bright orange. One washing may suffice when the urine contains a very small amount of sediment. The stains may be used singly, first staining with

fuchsin, washing in water and counter-staining with methylene blue. Leukocytes and epithelia cells stain distinctly, the protoplasm of renal epithelium usually taking a dark red color, that of squamous epithelium a light pink. Red blood cells may show a pale salmon color. Mucinous products are distinguished chiefly as filaments, agglutinations, showing their structure by longitudinal or spiral striations, and homogeneous casts. These forms merge into each other, there are no sharp dividing lines. Granular casts stain red and are thus more easily found than in the unstained sediment. Homogeneous casts staining red may be found in connection with granular casts. They are frequently of high density and refraction, assuming a waxy character. *Jour. A. M. A.*, March 18, 1911. (Abstract in *Archives of Diagnosis.*)

MOUNTING THE SEDIMENT.

Dry sediments obtained according to the method of Délépine may be mounted in Canada balsam as follows: place a drop of water on a cover-glass, mix with it a little of the dry sediment and let dry in air. Just as soon as dry, place face downwards upon a drop of thick balsam in the center of a slide and gently press down. This method mounts all crystals except sulphate, oxalate, and phosphate. The latter may be preserved in cells of varnish, made by "ringing" glass slides with shellac varnish, covering, and sealing with varnish. Instead of obtaining the sediment in a dry form, if it is desired to mount it after washing, the sediment should be kept in a little water for several weeks, then a drop of it mounted. Triple phosphate should be treated with ammonia instead of water.

Where Mueller's fluid is used, in order to mount the casts mix a drop of the sediment with a drop of the alcohol-glycerine-carbolic solution described above and preserve in a cell or examine under a cover-glass. Other organized sediments should be washed four or five times with the glycerine solution and then mounted in it as above.

In Cohn's method the specimens are mounted in glycerine.

When Farrant's fluid is used, the urine is mixed with 1 c.c. of 1 per cent. eosin or methyl violet, centrifugalized, and washed

by same until all urine is removed. One drop of the sediment is then mounted on the slide with one drop of the Farrant fluid.

For "ringing" slides proceed as follows: place a glass slide on a turn-table and make the cell by use of Bell's cement and a camel's hair brush. Allow the cement to dry thoroughly. To mount, place a drop of the prepared sediment within the cell and cover with a circular cover-glass of such size that its margin rests well on the ring of cement. Take up the excess of fluid from around the cover-glass by means of filter paper, care being taken not to admit air to the cell and also to remove all air bubbles that may be present. Return the slide to the turn-table and carefully cover the margin of the cover with the cement, so as to make the cell air-tight. Allow this layer of cement to dry and in two or three days apply another coat.

IDENTIFICATION OF CONSTITUENTS OF URINE SEDIMENTS.

☞ In order to identify objects seen through the microscope considerable experience is necessary, and progress without a teacher is necessarily slow, but by the process of reasoning from observation detailed in the following pages a certain amount of useful information may be obtained. The author insists upon the use of the 1/2 inch and 1/5 inch objectives for urine examinations, rather than the 2/3 inch and 1/6 inch as ordinarily provided for other medical work, dispensing with the cover-glass.

☞ It should first be borne in mind that large or highly colored conspicuous objects are most likely to be of extraneous origin. Beginners are usually attracted by hairs, threads, fibres, cracks in the glass, etc. Such colors as bright carmine, blue, and black are seldom characteristic of objects derived from the body. On the other hand, yellowish, reddish-yellow, brownish-yellow and greenish-yellow colors suggest derivation from the body itself. Again, absence of color in the object is still more suggestive of origin within the body, since epithelium, leukocytes, and many casts have no color. Even the red blood-corpuscles in urine, when seen under the microscope, may show but little color; again such objects as epithelium, leukocytes, casts, etc., are more or less faint and require good light and exact focussing to be seen

well, while crystals and extraneous objects are easily found and readily seen.

Things Easily Seen With a Low Power (150 diameters).—Extraneous objects (hairs, fibres, etc.) uric acid crystals, triple phosphate crystals, stellar phosphate crystals, large oxalate crystals, large casts, large epithelium, connective tissue shreds and bacteria masses (zoöglœa).

Things Requiring a High Power For Identification (500 diameters.) Blood corpuscles, pus corpuscles, most oxalate crystals, most epithelium, spermatozoa, individual bacteria, ammonium urate crystals, fat granules, fatty masses, most tube-casts, except the large granular and waxy, fat in casts.

Urine sediments are composed of organized and unorganized constituents. The former consist of casts, corpuscles, etc., the latter of mineral matter, salts of various acids, crystals, etc. Both classes may, of course, be present on the same slide. Organized constituents tend to be faint, pale, and more or less colorless; unorganized to be prominent and in some cases richly colored.

☞ In looking through the microscope using first the low power (150 diameters or nearly) consider whether the objects seen are (a) *crystalline, i. e.,* of definite geometrical form and strongly refractive of light, or (b) whether they are *shapeless and granular;* (c) whether they are *pale, roundish, elongated or irregular* in form; (d) whether they are *large* and *in some way striking* in appearance. Objects appearing as in (a) or (b) are likely to belong to the unorganized class; those appearing as in (c) of the organized and those as in (d) of extraneous origin, *i. e.,* hairs, fibers, cracks in the glass, etc.

Having decided that the objects are, for example, unorganized (crystalline or amorphous) we have to choose from the following:

Uric acid: crystalline in acid urine.

Sodium urate: crystalline in acid urine.

Urates (various): amorphous in acid urine.

Hippuric acid: crystalline in acid urine.

Calcium oxalate: crystalline in any reaction.

Calcium sulphate: crystalline in strongly acid urine.

Xanthine: crystalline in strongly acid urine.

Creatinine: crystalline in strongly acid urine.

Cystine: crystalline in feebly acid or acid urine.

Calcium carbonate: crystalline in feebly acid, neutral or alkaline urine.

Calcium phosphate: amorphous or crystalline in feebly acid, neutral or alkaline urine.

Magnesium phosphate: amorphous or crystalline in feebly acid, neutral or alkaline urine.

Ammonio-magnesium phosphate: crystalline in neutral or alkaline urine.

Indigo: crystalline in alkaline urine.

Cholesterine: crystalline, any reaction.

Leucine and tyrosine: crystalline, any reaction.

Hematoidin, bilirubin: crystalline, any reaction.

Melanin: amorphous, any reaction.

Some of these constituents are common, others rare, hence in attempting to identify them proceed as follows:

☞ A. CRYSTALLINE SEDIMENTS OF COMMON OCCURRENCE.

Urine Plainly Acid.—Expect to find amorphous urates, crystalline uric acid and crystalline calcium oxalate.

Urine Feebly Acid, Neutral or Alkaline.—Expect to find amorphous phosphates, crystalline calcium phosphates, crystalline calcium carbonate, crystalline ammonio-magnesium phosphate, crystalline ammonium urate.

Urine of Ammoniacal Odor.—Expect to find ammonio-magnesium phosphate and ammonium urate.

B. CRYSTALLINE SEDIMENTS OF LESS COMMON OCCURRENCE.

Having proved absence of crystals of common occurrence, next consider those of less common occurrence.

Crystals Having Color.—Extremely small yellow or red-brown rhombic tablets or needles alone or with amorphous yellow granules may be *bilirubin* or *hematoidin*. Blue rhombic crystals and rosettes of fine blue needles, mostly cohering in clusters in

alkaline urine, may be *indigo-blue;* rosettes of violet-red needles or violet-red rhombic crystals, *indigo-red;* yellowish, reddish or brownish large needles or prisms, the latter showing indentations with a high power, *hippuric acid* in acid urine.

Colorless Crystals.—In acid urine, colorless small lemon-shaped or whetstone tablets, *xanthine;* long, thin, colorless needles or in rosette form, in strongly acid urine, *calcium sulphate;* colorless or light greenish crystals in shape somewhat like those of uric acid, but seen with a power of 500 diameters to have striations both concave and radiating, *creatinine;* thin hexagonal tablets superimposed upon one another or closely contiguous, with a high power seen to have radii of faint greenish tinge or opalescent luster, less commonly highly refractive four-sided square prisms, *cystine;* large regular or irregular colorless, very thin rhombic transparent plates, some of which have notched corners and which overlap one another, *cholesterine;* branches of very fine needles in sheaf, tube or ball form, *tyrosine;* yellowish spherical masses of hyaline structure with both radial and concentric striations and which look large with a power of 500 diameters, *leucine;* large elongated highly refractive rhombic plates in feebly acid, neutral or alkaline urine, when concentrated, *magnesium phosphates.* See below for more common colorless crystals.

If crystals are found, consider the *size, color,* and *shape,* as follows:

Crystals Yellow, Red or Brown.—If large, likely to be *uric acid:* if smaller, *sodium* or *ammonium urate.* If uric acid, prisms, wedges, whetstones, rosettes, hexagonal plates, pointed bundles, barrels, blocks, etc. If sodium urate, fan-shaped clusters or needles (colorless). If ammonium urate, "thorn apples," yellow or red-brown spherules with or without spicules, often double, or in clusters, aggregations, or chains.

Crystals Colorless.—If faint yellow and small, may be uric acid; hexagonal crystals, or colorless, sodium urate in acid urine; colorless crystals in feebly acid, neutral or alkaline urine are, if large, *calcium phosphate, i. e.,* "stellar" star shaped, wedge-shaped formations with free single blades; or *triple phosphate* (ammonio-

magnesium phosphate) prismatic "coffin lid" crystals, the largest of all; small colorless, octahedral crystals in urine of any reaction are likely to be *calcium oxalate*, "letter envelope" crystals or dumb-bells, discs, and hour-glass crystals; small colorless spherules or dumb-bells in feebly acid, neutral, or alkaline urine are likely to be *calcium carbonate*.

Large Crystals.—Likely to be uric acid, stellar phosphate or triple phosphate.

Small Crystals.—Likely to be calcium oxalate, calcium carbonate or ammonium urate.

Identification of the various crystals and of the amorphous sediments may be completed by study of the particular appearance and properties of each constituent. For example, if the observer is in doubt whether he has found uric acid crystals or those of ammonium urate, careful reading of the following pages should enable him to differentiate by coupling the physical, chemical, and microscopical characteristics together.

The more commonly occurring unorganized sediments are those of uric acid, urates, calcium oxalate, and the various phosphates, which will be considered in this order.

SEDIMENTS OF URIC ACID.

Occurrence.—In acid urine, usually sharply acid.

Color and Appearance.—Crystals visible to the naked eye, looking like red-pepper grains, prone to cling to the sides and bottom of the glass, very heavy, falling quickly to the bottom of the glass when the urine stands. Best seen by the naked eye when the observer is holding the glass above the head and looking upwards. Of deep yellow or orange-red color, in some urines pale yellow.

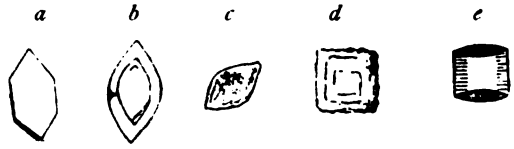
Crystalline Structure.—Primary form is a rhombic prism, and the crystals occur in various combinations or modifications of this form.

Solubility.—

1. Insoluble in dilute acids, as hydrochloric or acetic.
2. Soluble in fixed caustic alkalies (potassium hydroxide).
3. Converted into ammonium urate by ammonia.

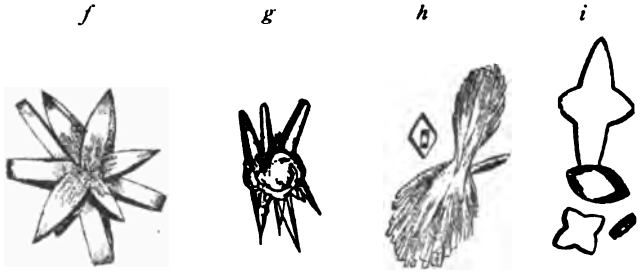
Microscopical Appearances.—Uric acid crystals (Figs. 37 and

38) have a rich yellow or orange color, or usually, at least, a pale yellow color. They occur as lozenge-shaped, rounded, barrel-shaped, compound, twin, cross, or rosette forms. In over-acid urines there are also seen spear-shaped or comb-and-brush-shaped crystals. They are easily seen with a low power (150 diameters) and look large with a high power (500 diameters). They may occur in enormous quantities, filling the entire field with beautiful forms of rich coloring.



☞ In some urines the crystals are colorless or of a pale lemon-yellow color and must, if hexagonal, be differentiated from cystine. (See Cystine.)

The crystals respond to the murexide test, etc. (See Chapter VII.) In carbolic acid poisoning the crystals are dark in color. The yellow color is due to urochrome, the red to this and uroerythrine. They may be stained, however, by other pigments, as bile, etc.



FIGS. 37 & 38.—Uric Acid (A): *a*, Colorless hexagonal; *b*, whetstone; *c*, rhomb; *d*, quadrate; *e*, barrel; *f*, rosette; *g*, sharp-pointed; *h*, sheave and rhomb; *i*, star, lozenge and whetstone.

Significance.—High acidity of the urine, poverty in mineral salts, low pigmentation, and high percentage of uric acid in form of urates in solution are factors which accelerate the precipita-

tion of uric acid in form of concretions or sediment. The latter is common in gout, rheumatism, fevers, diabetes mellitus, and chronic interstitial nephritis. The sediment is more common in the urine of men. ☞ Sharp-pointed crystals mean hyperacid urine, as in gout and rheumatism, but when accompanied by blood corpuscles, and especially blood shadows, suggest renal calculi. The occurrence of uric acid crystals in the sediment does not, however, necessarily signify an excess of uric acid total per 24 hours, since the other factors mentioned above may be responsible for its appearance.



FIG. 38.—Uric Acid (B): Miscellaneous forms.

SEDIMENTS OF CRYSTALLINE URATES.

Sodium Acid Urate (biurate).—When crystalline, this occurs in groups of fan-shaped clusters or colorless prismatic needles like calcium phosphate (Fig. 39). It occurs in amphoteric urine undergoing ammonical decomposition, and is very rare.

Ammonium Acid Urate (biurate).—This is found mostly in ammoniacal urine, appearing with a low power, as dark brown spheres, which, with a high power, may show fine sharp pointed spicules, so-called thorn apple crystals (Fig. 40). These crystals are found in urine which is originally rich in uric acid in solution, but which has undergone ammoniacal decomposition, hence the crystals are frequently seen along with triple phosphate in cystitis, stale urine, etc. If the sediment is not crystalline, but under the microscope appears to be composed of mossy looking masses without definite shape, the sediment appearing bulky to the naked eye, it is composed either of *amorphous urates* or *amorphous phos-*

phates, the one dissolved by warming, the other by dilute (50 per cent.) acetic acid.

Somewhat similar masses may be composed of *bacteria,* in which case instead of a bulky sediment we find a general haziness throughout the urine, which has a wave-like motion when disturbed by shaking.

SEDIMENTS OF AMORPHOUS URATES.

Occurrence.—In acid urine, composed of mixed urates of so-



FIGS. 39 & 40.—Urates: *a,* Crystalline sodium urate; *b,* amorphous urates; *c,* crystalline ammonium urate, "thorn-apple."

dium, potassium, calcium and magnesium, of which amorphous sodium urate is the most abundant. The urine is usually of high specific gravity.

☞ **Color and Appearance.**—Amorphous, reddish, granular sediment (Fig. 40), in color faint pinkish fawn color, reddish or brick-dust, forming what is known as the "brick-dust" sediment, which adheres so closely to the sides of the containing vessels, especially at the surface line of the urine. Scanty urines of high specific gravity are frequently turbid from presence of suspected urates. In the urine of children the mixed urate sediment frequently gives the urine a "milky" appearance with but a faint tint of color.

A pellicle of urates is frequently noticed on the surface of urines containing this sediment. The sediment is usually of a different color from the urine containing it, being deeper in hue. The red color is due to uroerythrin; the yellow to urochrome and also urobilin.

☞ **Tests.**—Dissolved by heat 50° C. (120° F.) or upwards and on addition of acetic acid to the solution uric acid crystals separate. If no acid is added, the sediment of urates reappears on cooling. It also responds to the murexide test, etc. (See Chapter VII.)

Microscopical Appearance.—Amorphous mossy granules (Fig. 40).

Significance.—The sediment occurs normally (1) when the urine is concentrated and strongly acid; (2) in a cold room; (3) when stale urine undergoes "acid fermentation." Temporarily the sediment is noticed in digestive disturbances, attacks of lumbago, and strain of any kind; more persistently in fevers, chronic renal hyperemia, gout, pneumonia and rheumatism; in the scanty high colored urine of dropsical patients. The sediment may be colored by the bile pigment and by the black pigment of carbolic acid poisoning.

Clinical Notes.—It has been said (1) that the presence of uric acid and of urates in the urine in the form of deposits is one of the most constant signs of functional derangement of the liver, and (2) that if, without errors of diet, a patient under forty habitually passes urine which soon deposits a pinkish sediment or which, though clear when voided, soon becomes thick and opaque, there is undoubtedly an undue tendency to produce uric acid. It is safer, however, to determine the ratio of urea to uric acid, and to note the quantity of indican before jumping at the conclusion that "uric acid" is the sole cause of the patient's ailment.

Rose-red urates are common in articular rheumatism, and are more highly colored (by uroerythrin) on the advent of pericarditis.

☞ **Laboratory Note.**—In looking for tube-casts in urine containing urate sediments, be sure first to dissolve the sediment by application of heat just short of boiling. (See Tube-Casts.)

This is done by immersing the bottle or tube in water heated to 140° F. (60° C.) The urate sediment will dissolve when the urine containing it is of a temperature of 120° F. (50° C.)

SEDIMENTS OF CALCIUM OXALATE (OXALATE OF LIME).

Chemical Constitution and Derivation.—CaC_2O_4

Occurrence.—Usually in acid urine, sometimes in alkaline. When occurring in profusion and in several forms, urine usually hyperacid.

Color and Appearance.—Light, easily moving sediment, usually of small bulk and colorless.

☞ **Crystalline Structure.**—Octahedra, made up of four-sided pyramids, situated base-to-base, as seen in their long diameters, or less commonly, ovoid or circular discs.

Chemical Tests.—

1. Soluble in hydrochloric acid, insoluble in acetic acid.
2. Insoluble in alkalies, as liquor potassæ.

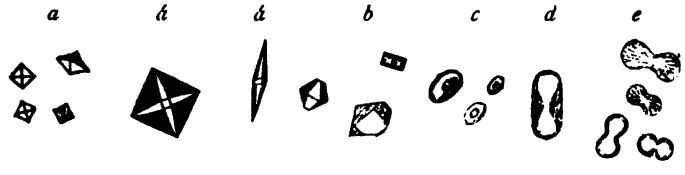


FIG. 41.—Oxalates: *a*, Small octahedra; *a'*, larger octahedra; *b*, imperfect octahedra; *c*, disks and spherules; *d*, hour-glass; *e*, dumb-bells.

Microscopical Appearances.—

☞ Common forms.—Small, colorless crystals (Fig. 41) seen with difficulty with a low power, 150 diameters, best studied with a high power (400-500 diameters), *octahedral* in form, highly refracting; have appearance of rear of a letter envelope, *i. e.*, squares crossed obliquely by two sharp lines. When small, the two lines form a bright spot at their crossing in the center. Edge view of the octahedra shows them as four-sided pyramids, base-to-base. Concretions of these crystals may occur as shown in the figure.

Calcium oxalate also ocurs in small circular crystals, sometimes smaller than blood corpuscles.

Rare forms.—Large octahedra, double octahedra ("twins"), discs and tablets with rounded corners. *The dumb-bell,* according to Heitzmann and others, is the disc seen in edge view. Uric acid sometimes crystallizes as dumb-bells, but they are brownish in color.

Calcium oxalate crystals are more easily found after any phosphates present have been dissolved by addition of acetic acid, or urates cleared up by heat.

The tablets with rounded corners are perhaps the rarest forms. The flat plates with parallel sides and rounded ends may look like superimposed sheets of mica. (Emerson.)

The largest octahedra may be mistaken for triple phosphates; the latter, however, is readily soluble in 20 per cent. acetic acid. Moreover, the large octahedra of oxalate occur with other forms of oxalate crystals, and, if the urine is strongly acid, there should be no doubt as to the constituent present. ☞ A more difficult differentiation is that of the small spheres from red blood corpuscles, since the crystals are biconcave. The oxalate spherules are unchanged by acetic acid, whereas red blood corpuscles are made smaller and paler.

Calcium oxalate forms are differentiated from calcium carbonate by insolubility in acetic acid.

Physiology.—The sediment is not of itself necessarily a sign of disease, but may be found after eating freely of fruits and vegetables. Spitzbergen apples, bananas, rhubarb and sweet carbonated drinks are conducive to the presence of these crystals in the sediment. Cabbage, carrots, beans, spinach, asparagus, sorrel, potatoes, onions, tomatoes, turnips, gooseberries, grapes, cresses, parsnips and sweets in general, fat meat, alkaline waters, fermented liquors, sparkling wines, cocoa, tea and coffee increase the amount of calcium oxalate in solution. The cause of the precipitation of the crystals is not understood except that diet rich in calcium and magnesium appears to aid. The sediment does not depend so much upon the total in the urine, as it does upon the presence of other constituents which tend to keep it in solution. The formation of crystals probably depends first on the amount of oxalic acid in solution in the urine; second, on the amount of

sodium dihydrophosphate (acid phosphate), which tends to hold calcium oxalate in solution, especially in warm urine, and third, upon the amount of magnesium in the urine, salts of which are supposed to have most to do with holding the oxalate in solution. Hence a urine rich in oxalate will deposit oxalate crystals if the amount of sodium diacid phosphate and of magnesium is small. Otherwise it may not do so.

Pathology.—Ogden distinguishes primary crystals of the oxalate, *i. e.*, those found within the body, from secondary ones formed after the urine is voided. Primary crystals are the large octahedra and the oval and dumb-bell forms. Secondary crystals are the small octahedra and perhaps all the very small forms. The primary crystals, if formed in the kidneys, are irritating; less so, if found in the bladder. Continued formation of the primary crystals in the kidneys shows a marked tendency to calculus formation, especially if accompanied by renal hemorrhage. ☞ Pain, frequent and painful micturition, concentrated urine, and even hemorrhage may follow the separation of these primary crystals in the kidney.

Oxalate sediments in urines of high specific gravity, 1026-1040, are found in many cases of diabetes mellitus, digestive derangement, hypochondria, melancholia, pseudoataxia, hepatic disorders, gout, diseases of the heart and lungs, and in general when oxidation is deficient. But the oxalate is not the cause of the disorder and the sediment disappears when the patient leads an out-door life, especially in the bracing atmospheres of the mountains. Neurasthenics and dyspeptics are prone to oxalate sediments.

The term "false Bright's disease" has been applied to a condition of extreme nervousness, emaciation, dry skin, constant pain or sense of weight across the loins, frequency of micturition, and other symptoms suggesting nephritis; microscopical examination of the urine, however, shows merely oxalate crystals.

Emerson comments on the fact that some life insurance companies regard oxaluria as an early sign of nephritis.

Swinburn, of New York, insists that he has relieved prostatic and urethral disturbances by treatment (calomel and salts fol-

lowed by dilute nitrohydrochloric acid after meals for a week or two) of oxaluria. He claims, following Bransford Lewis, that non-specific urethritis may be caused by oxaluria and in general that injurious effects may be exerted by both oxalate and uric acid, either by inciting disease where there has previously been health, or by rendering more serious and resistant to ordinary treatment other inflammations and disorders, (gonorrhœal, etc.).

THE VARIOUS PHOSPHATES.

The phosphates occurring commonly in the sediment are either crystalline or amorphous. Crystalline phosphates are (a) the ammonio-magnesium (triple) and (b) acid calcium (stellar), the former being very common, the latter somewhat rare.

SEDIMENTS OF AMMONIUM-MAGNESIUM PHOSPHATE OR TRIPLE PHOSPHATE.

Occurrence.—Usually in alkaline urine, sometimes in acid urine verging on alkalinity. It accompanies usually the amorphous carbonates and phosphates and often ammonium urate.

Chemical Constitution.—$(NH_4)MgPO_4.6H_2O$, called "triple" because of 6 H_2O forming, the third portion of the formula. It results from the formation of ammonia in urine due to decomposition of urea into ammonium carbonate and combination of the ammonium radical with magnesium phosphate, forming a double salt.

☞ **Color and Appearance.**—Phosphatic sediments of all sorts are whitish in color. When triple phosphate crystals are present in them they sparkle like minute diamonds when held up to a strong light. If the sediment is formed within the body, the urine is turbid when freshly voided.

Crystalline Form.—Triangular prism. The crystals belong to the rhombic system, but fern-shaped crystals occur in sediments artificially formed.

Chemical Tests.—A drop of 50 per cent. acetic acid on the slide dissolves the crystals, which are, however, insoluble in alkalies. The urine is likely to have an odor of ammonia and a rod moistened with hydrochloric acid fumes when held near the

urine. The urine on addition of acids is likely to foam vigorously (ammonium carbonate).

☞ **Microscopical Appearances.**—Triple phosphate (Fig. 42) occurs in the form of triangular prisms, complete and incomplete. The colorless crystals are easily seen with a low power (150 diameters) and with a high power are very large. The incomplete forms are seen alone in slightly alkaline urine, or in urine which having been acid is verging on alkalinity. The complete forms appear in profusion in strongly alkaline ammoniacal urine. The ends of the prisms are beveled. The term "coffin-lid" is used in describing them. Seen in edge view they appear as squares. Formed artificially they appear as star-shaped, feathery crystals. Both forms may be artificially prepared by adding a small lump



FIG. 42.—Phosphatic Crystals: *a*, Triple phosphate precipitated; *b*, triple phosphate spontaneously deposited; *c*, stellar calcium phosphate.

of ammonium carbonate to two or three ounces of urine and setting aside. The crystals are beautiful objects when seen by polarized light.

"The crystals are often very irregular, of a great variety of shapes due to rapid crystallization from concentrated solution, or especially when they are partially dissolved. They may occur as thin plates, some with beveled edges, some with square corners, some with rounded or beveled corners; or as wedges. But all give by refraction a greenish hue." (Emerson.)

The crystals are sometimes shortened, forming squares likely to be mistaken for octahedra of calcium oxalate. (See above.)

Physiology.—Normal urine, which has become stale and ammoniacal, deposits triple phosphate crystals. In freshly voided urine the sediment is pathological.

☞ **Pathology.**—Retention of urine is the cause of the presence of the crystals, usually in the bladder, less commonly in the pelvis of the kidney.

The sediment is due to ammoniacal decomposition of the urine within the body; urea is converted into ammonium carbonate which in turn gives up its ammonium to the magnesium phosphate present.

The sediment is found in obstructive diseases of the lower urinary tract; in retention of urine in the bladder or in the pelvis of the kidney. Hence common in diseases of the spinal cord (paraplegia), paralysis of the bladder, enlarged prostate, chronic pyelitis, and calculous diseases, stricture of the urethra, etc., etc.

☞ **Clinical Notes.**—In the absence of prostatic or spinal disease the occurrence of triple phosphate crystals with pus and blood in freshly voided urine suggests stone in the bladder; without pus and blood or with but few red blood-cells, stone in the kidney. In one of the author's cases, in which a young girl slipped a hair-pin into her bladder, triple phosphate crystals incrusted the hair-pin and appeared in the freshly voided urine, together with pus and blood.

☞ Uric acid calculus or other stone may become crusted with triple phosphate. When triple phosphate crystals are found in a case evidently of stone, it should not, therefore, be inferred that the stone is wholly phosphatic.

☞ Ammoniacal urine, with deposit of triple phosphate and pus, in elderly men is usually the forerunner of dangerous septic conditions resulting in so-called "surgical kidney."

☞ Triple phosphate crystals without pus or blood in freshly voided urine were a feature in the urine of one of the author's patients, who subsequently passed a phosphatic calculus following an attack of renal colic.

SEDIMENTS OF ACID CALCIUM PHOSPHATE (DICALCIUM PHOSPHATE, CRYSTALLINE CALCIUM PHOSPHATE, STELLAR PHOSPHATE).

Occurrence.—In pale copious urine, feebly acid or amphoteric in reaction and rich in calcium.

Color and Appearance.—Either in the whitish phosphatic sediment or in the light-colored small sediment of oxalate.

Solubility.—In 20 per cent. solution of ammonium carbonate and in acids.

Microscopical Appearance.—Pointed wedge-shaped formations, occurring as individual crystals or grouped together in more or less regularly formed rosettes (Fig. 42). The crystals are essentially crystalline rods, usually grouped in stellar, rosette, fan-shaped or club-shaped forms, but often in form of lances, wedges or small prisms, sometimes lying entirely unarranged. The crystals are colorless, but under the microscope they look dark toward the center of the clusters. They are easily seen with a low power (150 diameters). In faintly acid urine they resemble closely the acid sodium urate.

Differentiation.—From uric acid by solubility in weak (20 per cent.) acetic acid. From the acid sodium urate by rapid solubility in acetic acid and by absence of formation of uric acid crystals in the way of replacement. From triple phosphate by solubility in 20 per cent. solution of ammonium carbonate.

Physiology.—The appearance of crystalline calcium phosphate depends on an excess of calcium phosphate in a feebly acid urine. It is said that the sediment may be produced at pleasure by merely drinking lime water. According to some authorities it is more common in summer than in winter, and is then to be regarded as physiological.

Clinical Significance.—The sediment is said to occur more commonly in diseases of the joints. It also occurs under somewhat the same condition as triple phosphate.

SIMPLE PHOSPHATES (EARTHY PHOSPHATES.)

Chemical Constitution.—Basic phosphates of calcium and magnesium. $Ca_3(PO_4)_2$ and $Mg_3(PO_4)_2$. Neutral calcium phosphate, $CaHPO_4$.

Occurrence.—In alkaline urine. Neutral calcium phosphate in feebly acid, neutral, or alkaline urine.

☞ **Color and Appearance.**—The phosphatic sediment is light colored, usually dirty white; may occur as a flocculent turbidity in freshly voided urine, which settles rather slowly and is easily disturbed by shaking. Occasionally the sediment is so abundant as to

have a creamy white color, and may be mistaken by the patient for seminal fluid.

Chemical Tests.—

1. Urine more or less cloudy when freshly voided, but the sediment is not dissolved by heat. (Differentiated from urates.)

☞ 2. Readily dissolved by acids, even by 20 per cent. acetic acid.

3. Red litmus paper colored blue; does not become red again when dried, if triple phosphate is not present in large quantity. (Urine alkaline from fixed alkali.)

4. The urine does not smell ammoniacal, if triple phosphate is not present in great quantity.

5. A rod moistened with hydrochloric acid does not fume (if triple phosphate is not abundantly present).

NOTE.—Both simple phosphates and triple phosphates may occur in the same sediment, hence tests 3, 4 and 5 are of value only when triple phosphate is absent or present in relatively small amount, ammoniacal decomposition not having as yet taken place.

6. Urine effervesces on addition of acids but not so vigorously as when triple phosphate has been deposited by ammoniacal decomposition.

7. Urine when heated becomes turbid from further deposit of phosphates, but the turbidity disappears with effervescence when an acid is added. (Differentiated from albumin.)

Microscopical Appearances.—The simple phosphates are either amorphous in form or, less commonly, crystalline. (See Acid Calcium Phosphate.) If amorphous, the phosphate appears in the form of minute pale granules arranged in irregular mossy patches and sometimes mistaken for granular masses of organic matter. A drop of 20 per cent. acetic acid quickly dissolves this sediment on the slide. Amorphous phosphates under the microscope resemble amorphous urates (Fig. 40).

Physiology.—A slight sediment of amorphous phosphates occurring after a hearty meal is hardly regarded as significant, since the urine is frequently neutral or alkaline after meals.

Pathology.—The deposit is due to circumstances favoring increase of alkalescence of the blood, hence present, (a) when much

acid is lost from the stomach by vomiting or lavage or in diarrhœa; in (b) cases of debility with feeble respiration, convalescence from acute diseases, in flatulent dyspepsia, in the latter case due to accumulation of carbonic acid in the system and formation of alkaline carbonates. The condition is known as "phosphaturia," but the total P_2O_5 is not necessarily increased.

☞ *Clinically,* we find the sediment most often associated with intestinal indigestion, "flatulent dyspepsia." The sediment may be irritating and cause pain in the lower urinary tract. It is quite regularly accompanied by depression of spirits. Patients may imagine that they are losing seminal fluid. The sediment is frequently persistent following gonorrhœa.

In ammoniacal urine the sediment is accompanied usually by triple phosphate and sometimes by ammonium urate.

AMORPHOUS CARBONATES.

These occur along with the amorphous phosphates as already said and with practically the same significance.

CRYSTALLINE SEDIMENTS OF INFREQUENT OCCURRENCE.

The crystaline sediments occurring more rarely in the urine are those of calcium carbonate, magnesium phosphate, calcium sulphate, cystine, creatinine, cholesterine, hippuric acid, leucine, tyrosine, hematoidin, indigo, xanthine and melanin. Heteroalbumose and hemoglobin have also been found.

CRYSTALLINE CALCIUM CARBONATE.

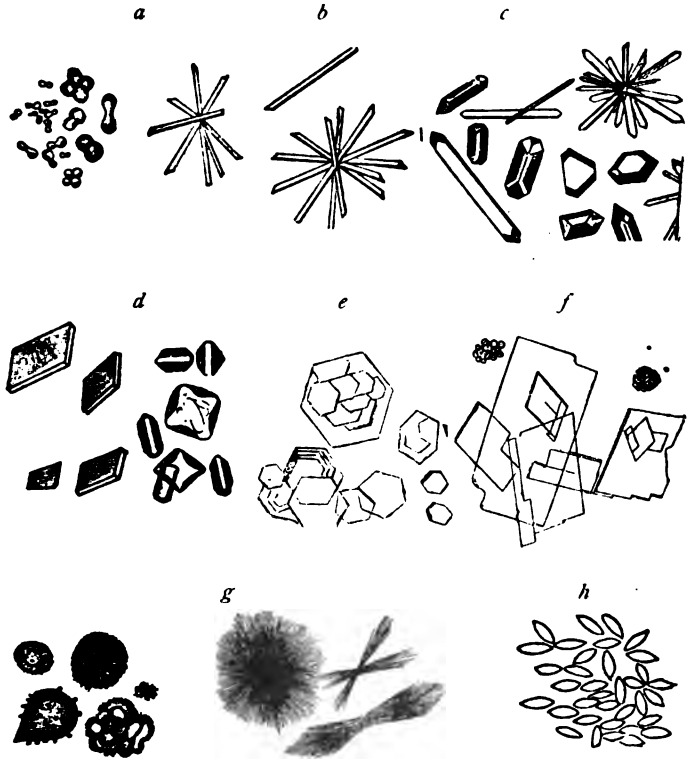
This is a rare sediment of whitish color and appearance like that of phosphates found in alkaline urine. It usually occurs with amorphous carbonate as above described. The crystals are in the form of granules, spherules and dumb-bells; soluble in acetic acid. Heitzman found them in rickets, bone caries and tuberculosis. (Fig. 43.)

CRYSTALLINE CALCIUM SULPHATE.

This salt, $CaSO_4,2H_2O$, is a very rare sediment occurring in concentrated urine of high acidity. It forms a whitish, heavy, dense sediment, difficultly soluble in hydrochloric and nitric acids

and in hot water. The crystals are insoluble in ammonia, alcohol and acetic acid.

The crystals are long and thin colorless tablets or needles (Fig. 43), usually in clusters, sometimes singly. The extremities are



FIGS. 43, 44 & 45.—Rarer Crystals: *a*, Calcium carbonate; *b*, calcium sulphate (strongly acid urine); *c*, hippuric acid (acid urine); *d*, basic magnesium phosphate; *e*, cystine; *f*, cholesterine and fatty masses; *g*, leucine and tyrosine; *h*, xanthine.

abrupt. It also ocurs in dumb-bell shaped amorphous masses.

The sediment, obtained by centrifuging, washing well with repeated centrifugalization, and dissolving in hot water gives a precipitate with barium chloride, insoluble in nitric acid.

The significance is unknown.

CRYSTALLINE MAGNESIUM PHOSPHATE.

These crystals have a composition of $Mg_3(PO_4)_2.22H_2O$, the normal salt of magnesium and phosphoric acid. They are very rare and occur in neutral, feebly acid or alkaline urines in which there is not enough ammonia to form triple phosphate. They take the form (Fig. 44) of large highly refracting rather long rhombic tablets or plates with beveled edges of great beauty. They are soluble in acetic acid and slowly in 20 per cent. ammonium carbonate, which makes them appear faint, and after some minutes eats away the edges. They occur in certain cases of dilated stomach with considerable vomiting and also after ingestion of magnesium compounds.

CYSTINE.

Occurrence.—A sediment seldom occurring, especially in strongly acid urine: when occurring is commonly in faintly acid, pale urine which on standing gives off odor of sulphuretted hydrogen as well as that of ammonia.

Color and Appearance.—Of pale lemon or dirty yellowish-gray color, often changing to green on standing.

Solubility.—

1. Insoluble in cold and hot water, ether, alcohol.
2. Insoluble in acetic acid.
3. Soluble in ammonia and in solutions of sodium and potassium hydroxides, as liquor potassæ, but insoluble in solution of ammonium carbonate.
4. Soluble in hydrochloric acid.
5. Soluble in solutions of oxalic acid.
6. Soluble in large excess of hot water.

Characteristic Test.—None.

Chemical Recognition.—Let urine settle, decant, filter sediment, wash latter with water, and (1) test on platinum foil. Cystine does not fuse but burns with bluish-green flame and without melting, while a sharp acid odor like hydrocyanic acid is evolved. (If in solution in the urine it may be precipitated by acetic acid, and its solubility ascertained in reagents mentioned under "Solubility" above.) 2. Heated with nitric acid it dissolves with de-

composition, and on evaporation, leaves a reddish-brown mass which does not give a purplish color with ammonia.

Microscopical Appearances.—Cystine (Fig. 44) may occur as hexagonal tablets superimposed upon or contiguous to one another, and which with a power of 500 diameters are seen to have radii, which are fine lines of secondary crystallization. In many the angles become worn off, approximating a circular form. The crystals may have a faint greenish tinge, or possess an opalescent lustre suggesting mother-of-pearl. Less commonly cystine occurs as highly refractive four-sided square prisms, whose sides are dark, when out of the direct line of vision, but brilliant white when presented vertically to the light.

Micro-chemical Reactions.—Differentiate from uric acid by its solubility in oxalic and hydrochloric acids; from triple phosphates by its solubility in ammonia.

Physiology.—Cystine sediments may be noticed in the urine for years, especially in young men, without impairment of the health of the individual, but they can hardly be called physiological.

Pathology.—Some families are prone to cystine sediments and calculi as others are to uric acid, hence it is probably associated with hepatic disorders. Cystine has been found in the urine of hepatic diseases, Bright's disease, chlorosis and acute articular rheumatism. Little is known about it.

It is said to occur more often in the urine of men than of women. Less than 200 cases have been reported. It occurs in some persons during their whole life and exposes them to much suffering from formation of calculi. In some cases the out-put of cystine is intermittent. One theory of this excretion is that, like alkapton, it is due to an individual variation in metabolism, *i. e.*, an inability on part of the organism to oxidize the cystine nucleus. Silver coins carried in the pockets of individuals subject to cysturia may be blackened, owing to the fact that it is sometimes eliminated by the skin, where it decomposes and sets free sulphuretted hydrogen.

Cystinuria does not appear to be connected with any other disease, but it has been known to exist in several members of the same family. It is rare in old age. Brieger finding cystinuria

associated with diaminuria claims that the condition is a result of a specific infection of the intestines.

CREATININE.

According to Chas. Heitzmann creatinine is sometimes found in the sediment of acid urine. The crystals are colorless or at most light greenish, in shape somewhat like those of uric acid, but seen with a high power show both concave and radiating striations.

Heitzmann found them in a case of uremia and regarded them as an unfavorable sign. Small crystals may be found, it is said, after excessive muscular exertion.

CHOLESTERINE.

Cholesterine, a fatty substance in constitution a monatomic alcohol, $C_{24}H_{44}O.H_2O$, occurs pathologically in urine. It crystallizes in large, very thin, transparent rhombic plates overlapping each other (Fig. 44). The sides and angles frequently appear broken. The acute angles are often from 76 to 87 degrees. In large quantities it appears as a mass of white plates with mother-of-pearl luster and a greasy feel. It may frequently occur as a film on the surface of the urine. It is always found associated with other fats, being formed from the fatty degeneration of pus-cells and breaking-down tissue. It is insoluble in cold alcohol but easily in hot, and also soluble in chloroform. If the crystals, under the microscope, be treated with a drop of 80 per cent. sulphuric acid solution a play of colors can be seen in which reddish tints are marked at first, and then a violet appears. Or if the crystals be treated first with dilute sulphuric acid and then with solution of iodine the colors will be violet, bluish-green, and, finally, a beautiful blue.

Clinically, we find the crystals in cases of pyelitis, pyonephrosis, echinococcus cysts of the kidney, catarrhal cystitis, rarely in nephritis, in cases of evacuation of an abscess into the urinary tract and in hydronephrosis. Its occurrence in chyluria is disputed.

HIPPURIC ACID.

This may occur in the sediment as colorless or milk-white four-sided semi-transparent prisms and rods with ends of two to four planes and whose sides sometimes show indentations. (Fig. 43.) It also occurs in clusters of very fine needles. The crystals are soluble in alcohol and ammonia, insoluble in acetic acid and hydrochloric acid. The sediment is probably due to fruits in the diet, but Heitzmann found it in diabetes mellitus. It occurs after ingestion of benzoic acid. The crystals are not always colorless as claimed, but, according to Hawk, are likely to be pigmented like uric acid. Hippuric acid does not respond to the murexide test and is much more soluble in water and in ether.

LEUCINE AND TYROSINE.

Leucine has not been found as a spontaneous deposit in urine, but only when the urine is concentrated by evaporation, especially when evaporated to one-tenth its volume and with addition of alcohol subsequently. The leucine crystals occur as yellowish spheres like oil-drops, which if more or less pure, have a hyaline or radiating structure, but are not highly refracting as claimed by some authors. If impure, they may be in spheres or masses without the hyaline structure. They are often arranged in masses and chains which merge together at the edges. They are insoluble in ether, and in cold mineral acids; soluble in alkalies. They somewhat resemble ammonium urate but lack the spiny projections.

Tyrosine occurs in the form of fine black appearing needles grouped in sheaves or in rosettes. They are readily soluble in acids, alkalies, and alkaline salt solutions, but difficultly soluble in cold water, strong alcohol, and ether. (Fig. 45.)

Leucine and tyrosine occur in acute yellow atrophy of the liver and in phosphorus poisoning. They have occasionally also been found in small-pox, severe typhoid fever, pernicious anemia, and leukemia.

HEMATOIDIN (BILIRUBIN).

These crystals occur as minute red-brown needles or rhombic tablets in cases of hemorrhagic nephritis, jaundice, acute yellow

atrophy, and in cancer fragments; they have also been found in pyonephrosis, after transfusion, in waxy kidney, scarlet fever, typhoid fever, and carcinoma of the liver with jaundice. Amorphous yellow granules of the same substance may also be found.

Some investigators claim that hematoidin is identical with bilirubin, others deny it. It is said to have been found in puerperal nephritis, phosphorus poisoning, and cancer of the bladder. It occurs commonly in urine containing bile and in cases of extensive hemorrhages into the urinary tract as in prostatics or in the case of those with cancer of the urinary tract.

INDIGO.

The breaking down of indoxyl compounds in the urine results at times in the deposit of crystals of indigo-blue. The sediment is fairly common in alkaline decomposing urine, which may show a bluish-red pellicle composed of crystals of indigo. The crystals may also be found at the bottom of the glass. They are in the form of rhombs and fine blue stellate needles, mostly cohering in clusters. It also occurs in amorphous flakes. It is more rare in acid urines than in alkaline ones. It is soluble in chloroform, imparting a blue color to it, but is insoluble in water.

Clinically, we find it in jaundice, abscess of the liver, peritonitis, pyelonephritis, intestinal obstruction, etc.

XANTHINE.

Xanthine has only been found two or three times in acid urine. The crystals are lemon-shaped or whetstone shaped (Fig. 45), somewhat like uric acid, but are soluble in hydrochloric acid and also dissolve when heated. They are also soluble in dilute ammonia. Xanthine may form a calculus, in which case the urine may contain crystals of it. Its significance in the sediment is not well understood.

MELANIN.

This is an extremely rare constituent of the sediment as it is usually in cases of melanuria held in solution. When in the sediment, it is found in the form of fine dark-brown amorphous scales or granules, occurring either in the free state or embedded in

epithelia, tube-casts, etc. The granules may be small and lumpy, much resembling carbon particles. They are soluble in boiling acids and in strong alkalies, insoluble in cold alcohol, ether, acetic acid, and dilute mineral acids.

HETERO-ALBUMOSE. HEMOGLOBIN.

Hetero-albumose has been found in the sediment once in crystalline form and once amorphous, according to Emerson. Hemoglobin may occur in hemoglobinuria in amorphous scales, plates, or casts.

Having finished consideration of amorphous and crystalline constituents of the sediment we next direct attention to the occurrence of fat.

FAT IN THE SEDIMENT (LIPURIA).

Fat is found in urine of any reaction and gives it a milky appearance, if in sufficient amount. Fat occurs physiologically in the urine during pregnancy, and following the administration or inunction of fatty substances. Microscopically (Fig. 52) fat appears as small, bright, highly refracting granules or globules requiring a high power,—300 to 500 diameters,—for recognition, except when very abundant or of extraneous origin from lubricants. The margin of the granules is dark and somewhat irregular. Ether dissolves them. Occasionally fat occurs in form of needles.

Fat occurs in greatest amount in the condition known as *chyluria*, in which it may form great tallow-like masses, but usually appears as a thin milk. On standing a cream rises or a fibrin coagulum forms. In such cases the urine contains albumin, sometimes cholesterine and lecithine, fibrogenic substances, hemialbumose and peptone. Shaking with ether clarifies this urine.

In lipuria, however, much less fat is present, and it may be necessary to extract the urine with ether and test the residue by the stain on paper, the odor of acrolein when heated, the blackening on long contact with one per cent. aqueous osmic acid solution, and the red color with Sudan III alcoholic solution.

Clinically, we find fat in the urine chiefly in subacute, diffuse,

glomerular nephritis (large white or fatty kidney); also when pus from an old abscess finds its way into the urinary passages and in pyonephrosis; also in constitutional affections associated with marked cachexia or dependent on systemic intoxication, as phthisis, long-continued suppuration, pyæmia, yellow fever, poisoning by phosphorus or by carbonic oxide, poisoning from external use of carbolic acid, chronic poisoning by turpentine, severe injuries to the bones, diabetes.

Diagnostic Hints.—

1. If large fat granules are abundantly seen in the sediment with the microscope, use of the catheter may be inferred.

2. When small fat granules are found, not due to extraneous matters, fatty degeneration of the kidneys is the condition, more certainly if fatty casts are also found.

3. Connective tissue studded with fat granules is probably derived from the kidneys.

4. In the urine of women, fat granules of sebaceous origin (smegma) may be seen with the microscope in vaginal epithelia, and in epidermal scales from the nymphæ. These are particularly noticeable in cases of vaginitis and vulvitis, as from masturbation in female children, but are not significant of the latter unless connective tissue be also found. (Heitzmann.)

☞ **Laboratory Note.**—Look for fat in the upper layer of the sediment after repeatedly centrifugalizing urine containing pus.

☞ **Clinical Notes.**—When an unusually large amount of fat is found in urine in cases of abscess, it is to be inferred that sufficient sloughing is going on to set free the oil of the fatty tissues.

After fracture of the bones, if the marrow be crushed, fat may appear in the urine or after crushing or tearing of either the subcutaneous fat, the liver, or of fatty tumors.

Among the various diseases in which lipuria has been found are diabetes mellitus, alcoholism, tuberculosis, adiposity, nephritis, certain mental diseases, pancreatic and cardiac diseases, and after various protoplasmic poisons.

CHAPTER XXVII.

ORGANIZED SEDIMENTS: BLOOD, PUS, AND EPITHELIUM.

Classification of organized sediments.

Red-blood corpuscles in the sediment: different appearances, normal and abnormal; removal of blood from the sediment.

Renal hematuria: in what diseases occurring.

Differential diagnosis in renal hematuria: acute, subacute, and chronic nephritis; amyloid disease; renal hyperemias; carcinoma of the kidney; tubercular kidney; cystic kidney; renal calculus; stone in the ureter; malarial hematuria; vicarious hematuria; essential hematuria; hematuria from distoma; renal traumatism; papilloma; other renal causes.

Relation of blood shadows to renal hemorrhage.

MacGowan's summary of the cause of renal hematuria.

Bladder hematuria: appearance of the blood; causes of the hemorrhage.

Differential diagnosis in vesical hemorrhage: prostatic hypertrophy; vesical calculus; gonorrheal diseases; growths; varicose veins; ulcers; parasites.

Hematuria in bladder growths: bilharzia hematuria; prostatic hematuria; abscess of the prostate; cancer of the prostate.

Urethral hematuria: causes; characteristics of bleeding from the anterior urethra and from the posterior.

Clinical notes on hematuria: clinical method of differentiation of renal hematuria from bladder hemorrhage; relation of symptomless hematuria to bladder tumors; hemorrhages in anilin dye workers; rupture of the bladder; hemorrhage from the posterior urethra; blood from the seminal vesicles.

Hematuria in general: without pain; with pain; beginning and ending suddenly; terminal; terminal with great pain.

Pus corpuscles (leucocytes) in the sediment: appearance; recognition; nuclei, granules, processes, etc.; hematoidin crystals, free oil, etc., in pus sediment.

Micro-chemical tests for pus corpuscles; use of Lugol's solution, acetic acid, guaiacum, etc.

Estimation of number of corpuscles (Posner); Ehrlich's triple stain (formula, etc.).

Clinical significance of pyuria: relation of age and sex to the diagnosis; significance of the amount of pus; color of the pus; admixture of blood with the pus.

Diseases in which pyuria occurs and with what quantity of pus; abscess of the kidney, renal tuberculosis, vesical calculus, renal calculus, gonorrheal conditions, prostatic diseases; streaky, glairy pus, brown pus, etc.

Differential diagnosis in pyuria; renal pyuria, surgical kidney, pyelitis, bladder pyuria; the pus in acute inflammation of the vesical neck; in prostatic hypertrophy; pus from the anterior urethra; pus in the urine of women.

The two-glass test; the cystoscopic test; the test by ureteral catheterization; the bladder-washing test; the exploration test and the instillation test.

Epithelium: properties of the sediment; classification by Heitzmann.

Epithelial lining of the uriniferous tubules, pelvis of the kidney, ureters, bladder, prostate, vesicles, ejaculatory ducts, vagina, and Bartholinian glands.

Determination of the source of epithelium by the size: relation in size of renal epithelium to the leukocyte; comparative sizes of ureteral and prostatic epithelium; pelvic epithelium; size and shape; bladder epithelium: size and appearance; urethral epithelium; vaginal epithelium.

Requirement in the operator for diagnosis by observation of epithelium.

Classification of epithelium according to shape: flat or squamous, cuboid or round, cylindrical, or caudate.

Diagnosis by shape of epithelium: gonorrheal conditions; prostatic diseases; vaginitis; ulcerations of the cervix; catarrhal endometritis; cystitis; deposit of uric acid in the renal pelvis; calculus impacted in the ureter; pyelonephritis; hemorrhage and ulceration in the renal pelvis; renal epithelia and the relation of them to the diagnosis of nephritis; epidermal scales.

The anatomical or organized sediments found in urine are various. We distinguish the following:

Corpuscles: Red and white.

Epithelium: From various parts of the genito-urinary and urinary tract.

Epidermal scales:

Tube-casts and similar formations.

Fungi and micro-organisms.

Spermatozoa.

Shreds: connective tissue, gonorrhœal, etc.

An important class of objects coming under this head is that of the red blood corpuscles.

RED BLOOD CORPUSCLES IN THE SEDIMENT (HEMATURIA).

Red blood corpuscles in the urine (Fig. 46) present different appearances. In general they are (a) normal, or (b) abnormal, the latter lacking the color, size and shape of normal corpuscles.

The normal blood corpuscles in urine are the same as those freshly obtained from the ear or finger, and always have more or less yellow color; are smaller than leukocytes, biconcave discs in shape, without nuclei and absolutely homogeneous, *i. e.*, without nuclei or granules. They measure $^1/_{3200}$ of an inch in diameter on an average, and, on careful focussing, the center and periphery alternate in brightness or shadow as the objective is brought near the slide or removed from it. Blood corpuscles require high power—400 diameters or more—for recognition, and when at all



FIGS. 46 & 47.—Blood and Pus Corpuscles: *a*, Blood corpuscles, usual appearance in urine; *b*, blood shadows from the kidney in fresh acid urine; *c*, cremated blood corpuscles in acid urine; *d*, pus corpuscles, usual appearance; *e*, pus corpuscles, irregular and provided with processes (in acid urine); *f*, pus corpuscles, swollen, as in alkaline or watery urine.

abundant the microscopical field shows ☞ an immense number of small roundish objects whose mass presents a rusty or green-yellow color. The normal red blood corpuscles soon undergo change in the urine, their edges becoming irregular or *crenated*, especially when the urine is rich in sodium chloride.

Abnormal blood corpuscles are those which have absorbed water, become swollen and lost color, *i. e.*, changed into colorless spheres and reduced in size to about two-thirds the normal or much less.

☞ These abnormal corpuscles are known as blood shadows, rings, of ghosts. The identification of these shadows in freshly voided acid urine is one of the most important measures in urinology.

Blood corpuscles in the urine (Fig. 47), if abundant, form a sediment varying in color from bright red to dark brown or almost black. When present together with pus, the sediment of blood settles on top of the pus. Albumin is always found in urine containing an appreciable amount of red blood corpuscles.

☞ The term ***hematuria*** is applied to the condition in which blood corpuscles, together with hemaglobin, are found in the sediment. (See Chapter XX.)

☞ **Laboratory Note.**—The presence of blood in large quantity in the sediment is an obstacle in the way of discovering pus and tube-casts in smaller quantity. The method of Ogden for removing the blood is an excellent one, as follows: allow the urine to settle thoroughly in a urine glass (or centrifuge it possible), then decant the supernatant blood-fluid and to the sediment remaining in the glass add a large volume of lukewarm water and a few drops of dilute acetic acid. Stir thoroughly with a glass rod, breaking up all clots, and allow the fluid to settle again. Repeat this process until the waste water is practically free from blood pigment. Finally let settle and examine. If now leukocytes are numerous, and especially if in clusters, suppuration is present as well as hemorrhage.

In rare cases large shreds of connective tissue from tumors have been found by the author after dissolving out the blood as above.

Renal Hematuria.—In nephritis, acute or chronic; also in renal hyperemias, in lardaceous disease, in renal abscess, in cystic diseases of the kidney, in hyatids, in stone, in rare instances in aneurism and embolism of the renal artery and thrombosis of the renal vein, in cancer of the kidney, in tuberculosis of the kidney, in malignant forms of acute infectious diseases, as small-pox, yellow fever, malaria, etc.; and in leukemia, purpura, scurvy, hemophilia, filariasis, and in distoma; also in poisoning by turpentine,

creosote, carbolic acid, cantharides and the new synthetic compounds; in cases of uterine and crural phlebitis; as a consequence of injuries, blows, or wounds, or indirectly from concussion.

Lastly, it is possible to find renal hematuria in presumably healthy kidneys, said to be due to paralysis of the vaso-constrictor nerves of the blood-vessels and consequent escape of the red blood-corpuscles.

☞ DIFFERENTIAL DIAGNOSIS IN RENAL HEMATURIA.

☞ In *acute nephritis* we find a history of infection together with dropsy, pallor, and uremic symptoms. There is scanty urine containing much blood and albumin, numerous tube-casts of the hyaline, yellow granular and leukocyte variety; also casts studded with red blood-corpuscles or composed of them.

In *subacute nephritis* the condition persists for months, *i. e.*, dropsy, pallor, and albuminous urine, with blood in some cases. Tube-casts in the urine are plenty, including large rust-colored granular casts; also fatty, dark granular, and, perhaps, waxy.

In *chronic nephritis* (interstitial) we find only occasional hemorrhages with polyuria, pale urine, cardiovascular changes and uremic symptoms.

In *amyloid disease* (waxy kidney) there may be hemorrhages as a result of extensive infiltration about the smaller blood-vessels. The urine, when free from blood, is likely to be clear with but little sediment, to contain much albumin and perhaps large waxy casts. A history of long-standing suppuration is usually obtainable.

☞ In *acute renal hyperemia,* as after surgical operations, there is scanty, bloody urine, but the blood soon disappears when the urine increases, and the albumin also lessens and disappears. In congestion due to venous thrombosis in the new-born it is claimed hematuria is present.

In *chronic renal hyperemia* the hemorrhage is slight, and yellow granular casts may be the only evidence of it readily observed. Some obstruction to the circulation as, *e. g.*, valvular heart disease, is evident.

☞ **Renal Carcinoma.**—Troublesome, profuse and repeated

attacks of bleeding. Persistent burning pain in the back, partially relieved by rubbing and by change of position. Renal tumor and cachexia; pus not abundantly present in the urine; albumin not in excess of blood; bleeding much more profuse at some times than at others, with weeks' or months' interval. The urine of digestion may be extremely blody.

☞ **Renal Tuberculosis.**—Urine contains, besides blood, more or less pus, broken down debris settling with difficulty; intermittent hematuria; usually no pain, but tumor may be present. Emaciation, temperature rise, etc., may be observed. In tuberculosis there is often, but not always, a profuse discharge of pus in the urine. The patient rises at night and voids bloody urine. In rare cases the bacillus tuberculosis is found in the urine. Guinea-pig inoculation, tuberculin tests, etc., may perhaps verify the diagnosis.

Cystic Kidney.—In cases of this rare condition, after blood appears in the urine, it is likely to be constantly present and to become large in amount. The clinical features of the case otherwise are those of chronic interstitial nephritis.

☞ **Renal Calculus.**—The amount of blood varies with rest and exercise, being most after exercise, and may disappear when the patient is at rest. A few blood corpuscles, especially shadows in fresh acid urine, may be the only urinary evidence. Albumin is usually small in amount unless nephritis exists. There may be a few hyaline or hyaloepithelial casts. Crystals may or may not be present, especially sharp, spiny crystals of uric acid. The patient is usually in good physical condition otherwise, or may present a history of "rheumatism." There may be a fixed pain in the region of one or the other kidney, or wincing on part of the patient on deep pressure over the kidney at a certain point. Pain may be present which follows the course of the ureter, sometimes with retraction of the testicle and often extending down the thigh. There may be a good deal of blood in the urine of digestion in some cases. Renal colic and positive X-ray results will confirm the diagnosis.

Stone in the ureter may be a cause of hematuria. Pain and frequency of urination are the features of the case.

Malarial Hematuria.—In suspected cases the plasmodium should be sought for. The symptoms may resemble those of vesical stone, but in addition chills, fever and sweat, beginning usually at the same hour of the day, suggest malaria.

Vicarious hematuria is possible in the case of women. Regular monthly hematuria has also been noticed in males.

"Essential Hematuria."—This term has been applied to symptomless hematuria of renal origin. The kidneys, when operated upon, show no gross abnormalities on either side. The terms renal hemophilia, angioneurotic bleeding, or neuralgic hematuria have been used in describing the condition. The hemorrhages are often profuse and the cystoscope shows them to be usually unilateral. A considerable proportion of them have been shown to be microscopically nephritic. Rovsing has shown that while nephritis of toxic origin is always bilateral, there are some cases of nephritis with hematuria which are instances of infectious origin, and that such cases may be unilateral and involve only a part of the kidney.

In these essential hematurias some are not constant, but appear regularly on exposure to cold or after unusual exertion, or at irregular intervals without cause. Cases of true renal hemophilia belong to families where there are bleeders, and are not properly in this category.

Finding of infected urine on the bleeding side is of much service in the diagnosis.

Hematuria from distoma hematobium is intermittent, and the ova are found when the hemorrhage takes place.

Renal Traumatism.—Laceration of the kidney may be followed by hematuria without external appearance of injury. There may be also pain in the loins, pyuria, and a typhoid condition, followed by death in a few weeks.

Renal Papilloma.—Hematuria may be due to this cause or to angiomatous degeneration of a papilla.

Other Renal Causes.—Adrenal disease and movable kidney are rare causes. The hemorrhage may be due to diet, drugs, hemophilia, or blood degeneration, as in variola, typhoid and malaria.

Ureteral Hemorrhage.—Due to stone, new growths or tuberculosis.

☞ **Clinical Notes.**—In the absence of symptoms pointing to the bladder or urethra, the finding of blood shadows in freshly voided acid urine is proof that the hemorrhage is renal *even if tube-casts cannot be found.* The urine is likely to be smoky in color and to deposit a brown or coffee-colored sediment, but in hemorrhage from the straight tubes or in profuse hemorrhage the color is brighter red, hence it is not safe to attempt differentiation from the color alone.

Clots are not so common in renal hemorrhages as in those from the bladder, yet large clots, tough and organized, have been found by the author in cases known to be cancer of the kidney.

Renal hematuria is sometimes noticed at the time of the highest temperature in typhoid fever, disappearing on the fall of temperature. (It goes without saying that blood in the urine of menstruating women should not be taken too seriously as it is not renal.)

MacGowan summarizes the cause of renal hematuria os follows: The kidney substance lacerated or torn by force; the mucous membrane irritated or torn by pressure of stone; deposits of tubercle in the cortex with congestion or in the pelvis with causation and formation of slough; malignant tumors infiltrating its tissues; angiomatous degeneration of a pyramid; disease of the adrenal; displacement; a shower of uratic or oxalic crystals; papilloma, multiple cysts, echinococcus, nephritis and obscure trophic changes. Also the effects of such drugs as turpentine, phenol and cantharides.

If injury is sufficient to cause hematuria, the capsule of the kidney will be torn. In movable kidney abundant hemorrnage occurs from time to time, usually painless and probably induced by pressure congestion from obstructed circulation.

Large clots are more common in renal cancer than in renal calculus. In cancer of the kidney, according to Garceau, hematuria was present in 75 per cent. of 90 cases reported by Denaclara.

The hemorrhage in cancer comes on insidiously and without warning. The patient may have a sudden desire to urinate, and notices that the stream is blood-red in color. Occasionally the attack is ushered in by a feeling of weight, or by a dull pain in the

lumbar region. Frequently, however, the bleeding is entirely painless. Bodily motion seems to have but little influence with reference to the bleeding. An attack is liable to come on at night as frequently as it does in the day time.

The duration of the hematuria is variable; some patients may not pass blood more than a few times during the whole course of the disease, others may have an attack lasting a few months only, and the bleeding does not return until towards the end of the trouble.

The amount of blood lost is very variable; at times it amounts to only enough to color the urine red, while at other times the amount of blood lost may be so large as to simulate a true unmixed hemorrhage. Blood casts of the ureter are not uncommon, and large clots of variable size and shape are also seen; in these cases the clotting takes place in the bladder. It is astonishing to see how well the patient stands the loss of blood, even if the hemorrhage has been going on for days or weeks.

HEMATURIA FROM THE BLADDER.

☞ The urine is usually alkaline, often ammoniacal, and thick from muco-pus, clots are commonly found, the blood is brighter red and not intimately mixed with the urine. Blood clots of irregular form and large size are from bladder; epithelia from middle layers of the bladder are quite commonly found. The ratio of day urine to night is not usually permanently or seriously changed in purely vesical hemorrhages.

Hemorrhage from the bladder may be due to the following; Cystitis, usually trigonal, edema of neck of bladder and interureteral fold, ulceration of projecting adenoma of the prostate (non-malignant), stone, tuberculous ulceration, simple ulcer, patchy gonorrhœal cystitis, telangiectasis of the posterior slope between the vesical outlet and the ureteral opening; new growths, simple and malignant, bilharzia, traumatism, as from sounding or cystoscopy.

DIFFERENTIAL DIAGNOSIS IN VESICAL HEMORRHAGE.

Blood in the urine in small quantities, together with pus, in the case of old men, is quite common, as in the cystitis from enlarged prostate.

Blood in cases of stone in the bladder is almost normal in appearance, and is passed mostly at the end of micturition. The hemorrhages are provoked by forced movement, walking, riding, etc. A few blood cells can most always be found.

In inflammation of the neck of the bladder, blood, in small quantity, may be passed at the end of micturition. History of recent gonorrhea, and presence of considerable albumin (½ to 1½ in the Esbach tube) will serve to distinguish from stone.

Profuse vesical hemorrhages occur in connection with bladder growths. It may be possible in such cases to find connective tissue shreds on dissolving the blood as above described and numerous epithelia from the middle layers of the bladder are likely to be present. An alternation of clear and bloody urine on the same day is never seen.

Rupture of varicose veins in the bladder may be a cause of vesical hemorrhage, especially in elderly patients; vesical hemorrhage occurs in ulcers and parasites of the bladder.

☞ **Laboratory Note.**—Blood shadows are quite commonly found in vesical hematuria with alkaline urine.

According to Ogden they may also be found in vesical hematuria when the urine is highly acid, but the author has not yet seen such a case without a history pointing plainly to the bladder.

In renal hemorrhages the blood is equal in amount, when the urine is voided into two glasses, while in cases of hemorrhage from the bladder it is usual to find more blood in the second glass and the bladder washings blood-stained, while in renal hemorrhage the bladder washings may be clear.

☞Fish-worm or pencil-shaped clots usually come from the ureter in renal hematuria, but occasionally they are of urethral formation, as in slow hemorrhages from villous growths of the bladder near the ureter, or from congested ulcerated verumontanum.

Hematuria in Bladder Growths.—Casper says that hematuria may be for years the only perceptible sign of a vesical growth. It is usually abundant, of long duration, and occurs irrespective of injury, violent efforts, or straining. It is very obstinate in

yielding to therapeutic measures and disappears without reason, either to recur in a few days or to remain absent for a long time. In some cases when the tumor is near the neck of the bladder the bleeding may be terminal only. When the hemorrhage is well nigh constant the condition is nearly always malignant.

In neoplasms of the bladder the urine is at first not mixed with blood or only slightly so, but towards the end of micturition it becomes more and more so and the last part may consist of almost pure blood.

In bladder growths the bleeding may be symptomless, as in papilloma, but in malignancy it may be painful. The hemorrhage is likely to follow compression or tearing of the tumor by forceful contraction of the abdominal muscles upon the bladder. When pain is present in carcinoma, it is likely to be constant, localized, and perhaps referred to the abdomen as well as to the bladder.

Bilharzia hematuria has been encountered in the United States among soldiers and others who have been in the tropics. The blood is usually at the end of urination and contains ova of the bilharzia. There is burning and itching in the perineum and frequency of urination. Membranous cystitis with hemorrhagic areas is present. The condition yields to formaldehyde antiseptics internally.

"Prostatic" Hematuria.—Hemorrhages occur in the course of prostatic hypertrophy and may be exceedingly profuse and uncontrollable, lasting for days. The hemorrhage is not, however, from the prostate, but usually from the bladder, though occasionally it may originate in the kidneys. Hematuria is a frequent symptom in prostatics and may be due to simple congestion, breaking of a blood vessel, from muscular strain or pressure, awkward efforts at catheterization or in expelling hard fecal masses. It is not necessarily to be regarded as a sign of malignancy. Severe and fatal hematuria may result from too rapid primary catheterization in cases of prostatic obstruction.

☞ **Abscess of the Prostate.**—Blood is found in the urine in this condition and may ooze from the meatus between urinations.

☞ **Cancer of the Prostate.**—In this condition blood may ap-

pear at the meatus in the intervals between urination or between catheterization, thus simulating urethral hemorrhage.

Urethral Hematuria.—According to Valentine and Townsend, in hemorrhage from the anterior urethra, blood may appear at the meatus or ooze or stream therefrom or may drip after urination. This bleeding is usually not associated with pain, nor is it forcibly ejected by the detrusors, as it is in terminal hemorrhage of posterior urethritis.

In an anterior urethral bleeding the first urine usually is bloody, the second may be clear, and the last blood-tinted or clear.

Anterior urethral hemorrhage may be due to external injury, such as that of "breaking a chordee" or misdirected attempts at coitus.

Anterior urethral bleeding may be produced by internal traumatism, such as badly managed instruments, or other foreign bodies introduced from without or descended from the bladder (stones, broken catheters, etc.).

It is usually due to gonorrheal conditions, chancre, warty growths, tuberculous ulcers or traumatism. Sometimes terminal hematuria may be due to granular patches in the bulbous urethra and not to posterior urethritis. (See below.) Stricture is a common cause.

It must not be forgotten, however, that in prostatic abscess and in cancer of the prostate blood may ooze out of the meatus between the acts of urination.

Hemorrhage from the posterior urethra usually begins with the end of urination and follows the act. Blood may be also admixed in the last urine or drip or be forced from the urethra after urination. Posterior urethral bleeding is usually associated with and followed by pain. Occasionally the entire quantity of urine is blood stained.

Hemorrhage from both urethræ most frequently occurs in peracute gonorrhea, after violent instrumentation, and injudiciously employed injections and irrigations. When due to gonorrhea, it must be treated by irrigations plus rest and according to the other directions usually given.

Hemorrhage from the posterior urethra into the bladder is recognizable by

(a) The blood-clots not being worm-like, as they usually are in bleeding from the ureters.

(b) The absence of that pain which ordinarily accompanies the end of urination in posterior urethritis, and by the entire urine being bloody, with large clots.

Cystoscopy often is necessary to determine whether blood comes from the badder or posterior urethra.

The macerated lining of posterior urethritis with erosions of the mucosa, and papillomata, are the most frequent causes of posterior urethral bleeding.

In the absence of gonorrhea, or of traumatism, or of a growth, persistent terminal bleeding with slight pain may indicate tubercular invasion of the posterior urethra.

Non-tubercular posterior urethral bleeding, if due to gonorrhea, generally yields to one hot intravesical irrigation; if due to traumatism, it may require the perineal crutch; if due to the presence of a papilloma, that must be removed; if superficial, injections of suprarenal extract may arrest it.

Clinical Notes.—Cumston suggests that to differentiate between renal and bladder hematuria an excellent method is to empty the bladder with the catheter and leave the latter *in situ;* then, pressing the bladder between a finger introduced into the rectum and the hand depressing the hypogastrium, if the bladder is the seat of the hemorrhage, blood will escape from the catheter, but if an abundant renal hemorrhage is present, it will flow out of the catheter without pressure as above.

Symptomless hematuria is, according to Wallace, in the majority of cases, the first symptom of vesical tumors.

Bladder tumors are common among those who work in aniline dyes, hence a sudden hematuria in such persons should suggest presence of a tumor.

In rupture of the bladder there is always hematuria.

Hemorrhage from the posterior urethra is usually due either to enlarged or inflamed veru montanum, to posterior urethritis, or to inflammation of the prostate or seminal vesicles.

Seminal Blood.—Blood from the seminal vesicles will be clotted and mixed with yellow objects and spermatozoa. If the spermatic fluid is bloody, the blood probably comes from the prostatic sinus.

HEMATURIA IN GENERAL.

Hematuria Without Pain.—Suggests new growths, especially if sudden, profuse and without apparent provocation.

Hematuria With Pain.—In infants, probably stone; in youths, gonorrhea and tuberculosis; in middle life, new growths; in old age, enlarged prostate and stone. In aniline dye workers, carcinoma.

Hematuria Beginning and Ending Suddenly.—Suggests neoplasm, especially if the blood is bright red.

Terminal Hematuria.—Suggests the bladder as source.

Terminal Bright Red Hemorrhage With Great Pain.—Suggests either vesical tuberculosis or calculus. Frequency, diurnal and nocturnal in the former; diurnal or on motion in the latter is noticed.

PUS CORPUSCLES IN THE SEDIMENT. PYURIA.

Recognition of pus by chemical means has already been described. Microscopically, we find *pus corpuscles* (leukocytes); these are about one-third larger than blood corpuscles, granular, and sometimes showing nuclei. The latter may be brought out by addition of acetic acid.

RECOGNITION OF PUS CORPUSCLES.

☞ Urine containing abundance of pus corpuscles deposits a dense white or greenish-white sediment. In less abundance the deposit is flocculent, granular, and "branny."

At least a trace of albumin is to be found in the urine, but seldom a large quantity. The precipitated proteins in the Esbach tube will usually settle below the figure 1.

☞ The corpuscles must be examined with a high power, 400-500 diameters. If at all abundant, the field is seen to contain a large number of small, colorless, roundish, well-defined objects, granular and sometimes exhibiting nuclei (Fig. 47).

In ammoniacal urine it is hard to see the individual corpuscles, which have broken up and coalesced to form a granular mass.

☞ In strongly dilute or alkaline urine they are much swollen, and have visible nuclei in the central portion, while the peripheral portions show only a small number of granules.

In acid urine they are small and either globular, or else irregular, sending out processes. The latter occur in the more obstinate cases of pyuria.

Solution of iodine in potassium iodide (Lugol's solution) colors pus corpuscles a fine yellow, while the nuclei appear darker and of a brown-yellow color.

There are usually several nuclei, two or more in every corpuscle; hence the term *polymorphonuclear leukocyte*. Occasionally those with but one nucleus occur. The nuclei are indistinct in fresh acid urine. In pus which has been mixed with the urine for some length of time the nuclei are more prominent and may fuse into a horse-shoe shape, especially noticed in old chronic suppurations. Fresh pus corpuscles may present processes and resemble caudate epithelium; the so-called ameboid movement is noticed when the slide is warmed slightly. The granules in pus may be coarse or fine. In some cases the corpuscle is almost without granules.

☞ All urine contains a few leukocytes. It is only when present in abundance that the term pus is applied to them.

Fat globules may appear in pus corpuscles and in some cases replace the whole cell. This is especially true in cases of long standing suppuration.

Dark-brown pigment deposits also occur in pus corpuscles, especially in chronic catarrhal cystitis. Small rust-colored hematoidin crystals are sometimes seen in the pus cells, and signify that some previous hemorrhage has taken place; in cases of chronic renal abscess large amounts of hematoidin are found mixed with the pus.

Free oil mixed with pus is usually more abundant in renal pus than in pus from elsewhere. The author's method of detection is to sediment the pus at low speed, decant the supernatant urine and sediment it again at higher speed, when oil globules, if present, will be abundantly found in the sediment.

Micro-chemical and Other Tests.—1. Add to the drop under examination a drop of Lugol's solution:—leukocytes are stained a deep mahogany-brown, while small round epithelial cells take on a light yellow.

☞ 2. Place a drop of 20 per cent. acetic acid on a clean slide, add to it a drop of the sediment under examination and mix well; the pus corpuscles swell, the granules dissolve, the body of the corpuscles becomes smooth, and the nuclei distinct. The body of the corpuscle soon disappears and eventually also the nuclei, but the latter much more slowly. The body of epithelial cells is not rendered thus faint or invisible.

3. In alkaline urine, if there is doubt as to presence of pus, acidulate with acetic acid, filter and treat filtrate with fresh tincture of guaiac. A blue tint on the inner surface of filter shows pus.

4. The number of leukocytes, according to Posner, may be estimated by placing a sheet of newspaper under a beaker and pouring into it well-shaken urine. If type cannot be read when the layer of urine is from 0.5 to 1 c.m. high 40,000 leukocytes per c.c. of urine are present; if it can be read when 6 c.m. high 1,000 leukocytes. From each 100,000 leukocytes per 2 c.c. of urine 0.1 per cent. albumin may be expected.

5. Ehrlich's triple stain: the number of nuclei in the leukocytes and the character of their granulations can be readily determined by use of Ehrlich's triple stain. This stain may be prepared as follows:—take 5 c.c. of a saturated aqueous solution of methyl green, 10 c.c. of methyl orange or orange G. and 2 c.c. of acid fuchsin, add 40 c.c. of water to each and then mix. The nuclei of pus corpuscles stain intensely blue with the Ehrlich stain.

Another formula for this stain is the following:—Saturated aqueous solution of orange G. (Grübler), 120-135 c.c.; saturated aqueous solution of acid fuchsin, 80-165 c.c.; saturated aqueous solution of methyl green, 125 c.c.; glycerin, 100 c.c.; absolute alcohol, 200 c.c.; water, 300 c.c.

Diagnostic Hint.—The nuclei of the pus corpuscles stain with greater difficulty in tubercular lesions than in others.

☞ **Clinical Significance.**—The sex of the patient has much to do with the significance when only a small amount of pus is found, which in women is more commonly of labial or vaginal origin, *e. g.*, as in leucorrhea and vulvitis. In the case of women, therefore, it is well to use vaginal douches, tamponing, or the catheter in order to exclude positively the generative tract as the source of the pus. In young men pus in the urine is usually of gonorrheal or tubercular origin. In middle-aged men it suggests stone and in old men prostatic disorders.

The amount of pus is of significance:—the sudden appearance of a large amount of greenish pus suggests the bursting of an abscess in the kidney or near the urinary tract, or the sudden removal of some obstruction in the kidney-pelvis or ureter. An intermittent discharge of pus suggests an obstruction in the ureter, as in hydronephrosis with suppuration (pyonephrosis); also a perivesical collection of pus (as in some cases of salpingitis) which has opened into the bladder may give rise to intermittent pyuria.

The color of the pus is of some significance, greenish pus in abundance being significant, according to Ogden, of abscess of the kidney, bursting of an abscess into the urinary tract, or pyonephrosis.

☞ The admixture of blood with the pus is of help in the diagnosis:—a considerable amount of pus and more or less blood points to renal tuberculosis, or vesical calculus; smaller or a moderate amount of pus tinged with blood to renal stone.

As regards diseases we find in abscess of the kidney a large amount of pus; in renal tuberculosis a large amount of pus; in vesical calculus a large amount of pus; in renal calculus a small or moderate amount of pus; in gonorrheal conditions a small amount of pus; in prostatic diseases a moderate or small amount of pus except after rupture of prostatic abscess or in cancer. Too much reliance, however, should not be placed upon diagnosis from the quantity alone. Sticky, glairy pus is in most cases due to cystitis or bladder conditions, but occasionally it is found in suppurative pyelonephritis with alkaline offensive urine. In the latter case the amount of albumin is large, in the former small.

Acid urine with "branny" pus and frequent painful micturition suggests posterior urethritis, a common sequel of gonorrhea. The amount of pus is not large, but the amount of albumin may appear large when the tenesmus is great.

☞ When the pus is from the kidneys, the patient is likely to be sensitive to pressure over the region of the kidneys.

Leukocytes microscopically are more abundant in acute nephritis or in acute exacerbations of subacute and chronic cases than in the latter. In simple renal hyperemia leukocytes are never plenty.

☞ **Differential Diagnosis in Pyuria.**—Pus from the kidneys is usually found in acid urine together with casts and renal epithelium. It settles quickly on standing, and is flocculent. The patient is usually sensitive to pressure over one kidney or the other, and may show presence of a tumor. When pus is retained in the calices, chills are often present. If the pus is only in the pelvis of the kidney, casts will be absent or scanty, and albumin not large in amount, below 1 on the Esbach tube. Frequent urging to urinate will not be a persistent symptom, though it may be complained of for a time.

☞ In suppurative pyelonephritis (surgical kidney) peculiar **casts of cocci** are found, extremely few in number, but characteristic. They look like short granular casts, but with a high power the coccus appearance is recognizable. The patient shows grave constitutional symptoms. In pyelitis the urine is generally acid and the microscope shows the pus cells in clumps.

The amount of pus in simple pyelitis may not be large. Free oil may be found in renal pyuria. A "cloudy pyuria" is a characteristic of chronic pyelitis, with frequency of urination at night.

☞ When pus is from the bladder, the deposit is glairy and sticky if the urine is ammoniacal, and there are found triple phosphate crystals with large, flat, and often round epithelia.

Frequent and more or less painful micturition is the rule in such cases.

☞ In acute inflammation of the neck of the bladder the urine is acid, and it may appear that the pus is from the kidney, but al-

bumin is fairly abundant, perhaps settling to 1, or higher in the Esbach tube, with severe pain, tenesmus, and frequency of urination and with a gonorrheal history.

☞ When the pus is due to prostatic hypertrophy, the sex and age of the patient and the discovery of an enlarged prostate with the finger per rectum are usually sufficient for diagnosis.

Pus from the anterior urethra is usually very dense and occurs in threads or shreds; if gonorrheal the color is usually yellow. It can be expressed from the urethra between urinations.

☞ "Gonorrheal threads" appear as white cottony masses in the urine and are composed of mucus, leukocytes, and urethral epithelium. The more numerous the leukocytes the more recent the case.

Pus in women from vaginitis or vulvitis should not be difficult of recognition, but we have known mistakes to occur. (See above.)

☞ Pus from the uterus may burrow into the urinary tract and appear in the urine. The diagnosis is then impossible without gynecological aid unless clever recognition of epithelium is available.

The Two-glass Test.—When the acid urine is voided into two glasses if the case is one of posterior urethritis the urine in the second glass may be clear, but sometimes is cloudy.

In pyelitis the urine in the second glass contains more pus than the symptoms referable to micturition (pain, tenesmus, frequency) account for. In anterior urethritis when the urine is voided into two glasses, the first glass is cloudy.

Absolute reliance should not be placed upon this test, as a second clear portion of urine free from shreds does not rule out with certainty the presence of posterior urethritis with its complications, prostatitis and vesiculitis.

The Cystoscopic Test.—If one or both of the ureteral orifices are inflamed and everted, and if the urinary jet is cloudy, the pus is from the ureters or pelvis of the kidney.

"The glycerin-like stream of health," as Fenwick calls it, is replaced in pyelitis by a muddy current of light straw to dark yellowish color.

Catheterization of the Ureter.—If pus is obtained in urine catheterized directly from the kidney, the diagnosis of pyelitis is rendered certain. Occasionally, however, the operation is unsuccessful and a urine free or almost free from pus is obtained, when much pus would be expected.

The entire length of the ureter may be tested and if clear urine is obtained at some point high up along the ureter and cloudy urine below it, a clinical diagnosis of ureteritis without pyelitis is possible except in the rare condition of double ureter with single opening into the bladder.

The Bladder Washing Test.—In washing the bladder when the return flow becomes quickly clear but soon clouds again, pus from the renal pelvis is most likely present.

The Exploration Test.—Exploration of the bladder with sound or segregator may reveal sensitiveness, when the pus comes from the bladder, and the segregator will show an equal quantity of pus from each side. When the kidney is involved, the bladder is painless, but the ureter is painful at its point of entrance into the bladder.

The Instillation Test.—A few drops of a 1:1000 methylene blue solution placed in the anterior urethra will stain all filaments, shreds, etc., coming from there, so that if, afterwards, unstained ones are voided they are from the posterior urethra or beyond.

EPITHELIUM.

Epithelium is the normal product of mucous surfaces and occurs in greater or less amount normally in all urine, especially in that of women, in which it may form a whitish flocculent sediment insoluble in acetic acid and not dissolved by warming the urine.

The diagnosis of certain urinary conditions can be made with positiveness by the identification of epithelium in the urine.

According to Dr. Chas. Heitzmann and his son, Dr. Louis Heitzmann, the following is true in regard to epithelia:

There are only three distinct forms of epithelia: first, flat or squamous; second, cuboidal; third, columnar or cylindrical. In health only a few flat epithelia can be found in the urine. As

soon as these become numerous, and as soon as cuboidal or columnar epithelia, no matter how few, are found, we have positive evidence of a diseased condition. Flat epithelia are more or less irregular in outline, exhibiting a broad front surface, although when seen in edge view they are narrower and somewhat spindle-shaped. Cuboidal epithelia are frequently seen as round formations in urine having about the same diameter in all directions. Columnar epithelia are conical or even caudate and elongated in one direction. All epithelia are granular and nucleated, posessing one or more nuclei; the latter, however, need not always be visible but may have dropped out, leaving a vacuole. Secondary degeneration may also take place, especially fatty degeneration; in these cases varying numbers of highly refractive, glistening granules and globules are found in the epithelia replacing the original granulation. The character of the granulation in the different epithelia likewise varies, some epithelia being finely granular, others of medium, and others of coarse granulation. As a rule, the flat epithelia appear more finely granular and paler than the others.

In the different organs, the epithelia lining them are present either in one layer or in a number of different layers; in the former case we speak of a simple epithelial lining, in the latter of stratified epithelial lining. In the majority of organs having a stratified epithelium all three forms of epithelia, that is, the flat, cuboidal and columnar are present. The flat variety composes the outer or upper layers; the cuboidal the middle layers; the columnar epithelia the deepest, one layer only. So-called pseudo-stratified epithelium may also be present, and here we usually have a number of layers of one form only. Where only one layer is present, this may be either of the flat, cuboidal, or of the columnar variety. Addition of acetic acid to the sediment containing epithelia causes the granulations to become indistinct and the nuclei to stand out. The various organs of the urinary tract are lined with epithelia as follows:

Uriniferous Tubules.—Simple epithelial lining of cuboidal epithelium in the convoluted tubules, flat in the looped tubules and columnar in the straight collecting tubules.

Pelvis of the Kidneys.—Stratified epithelia in disease, cuboidal and columnar, the majority caudate, pear-shaped or lenticular, usually decidedly irregular.

Ureters.—Stratified epithelia in disease, cuboidal and columnar, round, globular, oval and slightly irregular.

Bladder.—Stratified epithelia, normally flat epithelia in small amount; in disease more numerous together with cuboidal or columnar.

Prostate, Seminal Vesicles and Ejaculatory Ducts.—Stratified epithelia; both cuboidal and columnar. The cuboidal resemble ureteral epithelia. Those from the seminal vesicles are always columnar and more or less irregular. Those from the ejaculatory ducts are large, slender columnar formations, ciliated when *in situ*.

Vagina.—Stratified epithelia, resemble the bladder epithelia but are larger.

Bartholinian.—Stratified epithelia resemble the prostate.

The uriniferous tubules alone are lined by simple epithelium, all the other urinary organs having a lining of stratified.

A diagnosis can not be made from the shape alone of epithelia found in the urine. It is the **size** which determines the locality whence the epithelium is derived. It is not possible to determine the source of all epithelium found in urine, but that of the greatest number found can always be decided upon with proper care and study.

The Comparative Size of Epithelia.—Determine, first, in every sample of urine the presence of leukocytes, *i. e.*, the smallest granular corpuscles present varying in size only to a small degree. As soon as formations distinctly larger are found, about one-third as large again in diameter, these are kidney epithelia. If they are round the convoluted tubules are the location whence they are derived; if somewhat elongated and narrower they are from the straight tubules, the latter only in severe inflammations.

Next in size to kidney epithelia are those from the *ureter*. These have about twice the diameter of the leukocyte in a given case, those from the upper portion being larger. They resemble in size the epithelia from the prostate, so far as the cuboidal epi-

thelia are concerned, but the columnar epithelium is quite different, the ureteral columnar epithelium being caudate or pear-shaped, considerably elongated and perhaps somewhat irregular. In traumatism, or deep-seated pathological conditions, suppurations, ulcerations, hemorrhages, impacted calculus, tumor, severe destructive processes or injury from ureteral catheter, these caudate epithelia are in evidence.

The recognition of these caudate ureteral epithelia is a matter of considerable importance. They may roughly be likened to the head of a young chicken, the nucleus representing the eye and the caudate portion the beak, but since other formations also have this general likeness, the **size,** *i. e.,* twice the diameter of the leukocyte, and the considerable degree of elongation, are necessary to observe. Epithelium from the kidneys and pelvis as a rule accompany ureteral epithelium. Of the same size as ureteral is the *prostatic epithelium.* Cuboidal epithelium from the prostate is identical with ureteral, but the columnar epithelium differs and is almost always present along with the cuboidal. In the absence of epithelium from the kidney and renal pelvis, or if the latter are present only in small amount, the differentiation is easy, especially when the clinical history is positive.

Next in size to ureteral epithelium is that from the **kidney pelvis,** which is partly round but mostly irregular. The round ones are considerably larger than similar formations in the ureter, *distinctly nucleated* and usually of medium or coarse granulation. The majority of pelvic epithelia are usually caudate, pear-shaped or lenticular, though at times quite irregular. Their transverse diameter is a little greater than that of a leukocyte, while their long diameter is three or four times the transverse. The round epithelia are three or four times the diameter of the leukocyte.

Larger than pelvic epithelia and never as irregular or caudate is *bladder epithelium.* These in number are never found in urines obtained by the ureteral catheter. The flat cells are from the upper layer of the bladder and are largest in the vicinity of the neck.

The flat cells are found in small quantity in health. The round or cuboidal epithelia are always indicative of disease.

Epithelia from the surface of the *urethra* cannot, as a rule, be differentiated from that of the upper layers of the bladder and have no significance. The cuboidal and columnar epithelia are both more or less irregular and large except in the *pars prostatica*, where large, round, finely granular, pale formations exist, usually found along with prostatic epithelia, in size similar to the round from the renal pelvis, but always pale and finely granular.

☞ Largest of all epithelium in urine is that of the *vagina*. The flat are of no significance, being found even in the urine of little girls. The cuboidal are abundant in cases of *vaginitis* and the columnar only in intense deep-seated conditions of disease.

The following illustration of L. Heitzmann shows the distinction between the various epithelia as to size and shape.

It is evident that diagnosis of the location of epithelium should be attempted only by those whose eyes are capable of appreciating **size relationships**, *i. e.*, those whose power of measurement by the eye alone has been cultivated. It stands to reason, therefore, that a considerable number of physicians lacking this particular training of the eye cannot avail themselves of the help in diagnosis afforded by observation of the epithelium in urine. Our great authority on epithelium is Dr. Louis Heitzmann, of New York, whose study is shown in Fig. 48.

According to size and shape the various epithelia may be arranged from smaller to larger as follows:

Flat or Squamous.—Cervix uteri, female urethra; male urethra; in fossa navicularis, bladder, vagina. The approximate relative diameter compared with the leukocyte taken as a unit is stated by Saxe to be as follows: cervix, 3 to 5 +; male urethra in fossa navicularis, 5; female urethra, 3 to 5; bladder, 5 to 7 +; vagina, 7 to 8 +.

Cuboid or Round.—Convoluted tubules, prostate (acini) and ureter; renal pelvis, cervix uteri, urethra, bladder, vagina. Diameters compared with the leukocyte: kidney, 1½; prostate and ureter, 2; pelvis, 3 to 4; cervix, 3 to 5 +; urethra, 3 to 5; bladder, 5 to 7 +; vagina, 7 to 8 +.

Cylindrical or Caudate.—Kidney straight tubes, prostate (ducts), ureter (deepest), seminal vesicles (irregular), renal pel-

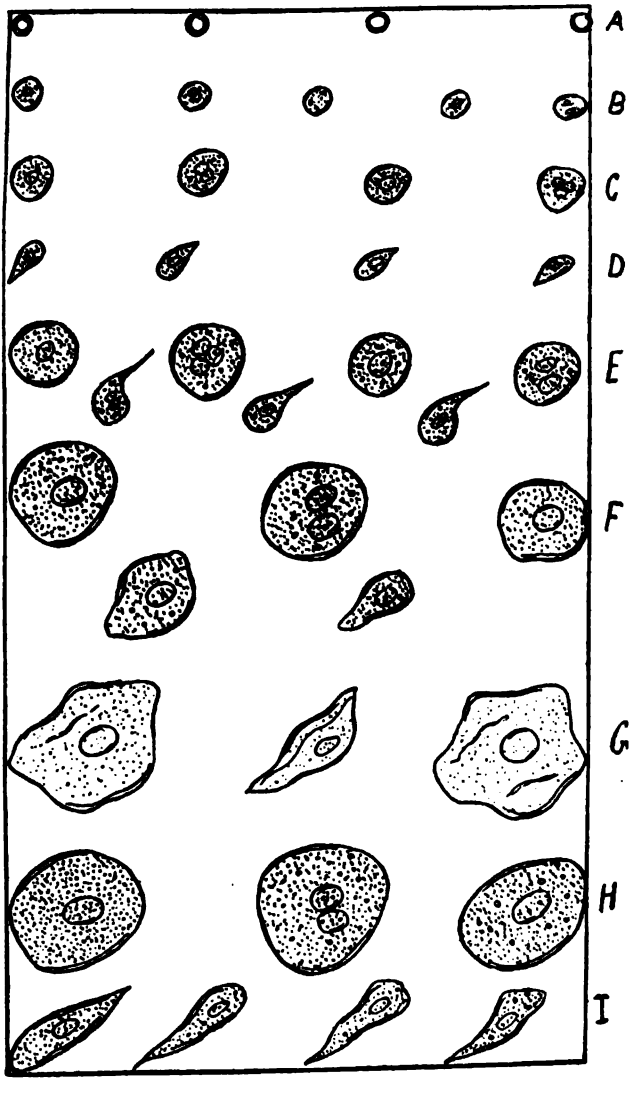


FIG. 48.—Diagram showing comparative sizes of Corpuscles and Epithelia. Magn. = 450.

A, Red blood corpuscles. B, Pus corpuscles. C, Epithelia from convoluted tubules of kidney. D, Epithelia from straight collecting tubules of kidney. E, Epithelia from ureter. F, Epithelia from pelvis of kidney. G, Epithelia from upper layers of bladder. H, Epithelia from middle layers of bladder. I, Epithelia from deepest layer of bladder. (Louis Heitzmann in *Archives of Diagnosis*, April, 1908.)

vis (common type), cervix (mucosa), urethra (more frequently male), ejaculatory duct (ciliated), bladder (deep layers), vagina (deep layers).

Kidney, 1½; prostate and ureter, 2; vesicles, 2½; pelvis, 3 to 4; cervix, 3 to 4; urethra, 3 to 5; ejaculatory duct, 5 +; bladder, 5 to 7 +; vagina, 7 to 8 +.

Diagnosis by Epithelium.—In urine of males:

The largest *columnar* epithelia from the urethra occur in deeply-seated gonorrheal inflammation, and in ulcerations, which often lead to formation of a stricture.

Cuboidal epithelia, somewhat smaller than the average cuboidal epithelia of the bladder, come from the prostate in catarrhal prostatitis (young men) and hypertrophy of the prostate (men over 40).

Ciliated columnar epithelia, distinctly surpassing in size those from the mucosa of the uterus, indicate slight catarrhal inflammation of the ejaculatory ducts. They are rarely seen ciliated, as the cilia break off very easily; delicate parallel rods in the interior indicate original ciliation.

In urine of females:

Large, flat, vaginal epithelia in number, and especially when accompanied by cuboidal, indicate catarrhal vaginitis. The largest cuboidal and columnar epithelia are observed in cases of intense, deeply-seated or ulcerative vaginitis.

Flat cuboidal epithelia, smaller than vaginal, and, as a rule, feebly granular, often with offshoots, are found together with leukocytes, red blood corpuscles and shreds of connective tissue in ulcerations of the cervix uteri.

Delicate columnar ciliated epithelia from the mucosa of the uterus, accompanied by ciliated pus corpuscles, indicate catarrhal endometritis.

In the urine of *both sexes*:—flat epithelia of the bladder in small numbers and without pus corpuscles are normal.

Flat epithelia in larger amount with leukocytes and cuboidal epitheria, the latter exhibiting endogenous new formation of pus corpuscles, indicate acute catarrhal cystitis.

If the cuboidal epithelia largely out-number the flat, or are

scanty in comparison with the larger amount of leukocytes, and especially if some of the latter are pigmented dark brown, the case is one of **chronic cystitis.** Clusters of uric acid crystals in freshly voided urine, together with caudate epithelia smaller than bladder cuboidal, indicate **deposit of uric acid in the renal pelvis.**

Caudate epithelia of moderate size, *i. e.*, twice the diameter of the leukocyte in cases without prostatic history, signify ureteral location and point to **position of calculus (impacted in ureter), source of hemorrhage or location of tumor.** These epithelia are found also as a result of injury from the ureteral catheter. They are almost always accompanied by renal and pelvic epithelia.

Pelvic epithelia together with renal from the uriniferous tubules indicate pyelonephritis; with red blood corpuscles and shreds of connective tissue, hemorrhage and ulceration in the kidney pelvis.

Renal epithelia together with leukocytes signify chronic interstitial nephritis, even if tube-casts are absent. The larger number contain fat granules and fat globules.

Renal epithelia with leukocytes and tube-casts, especially granular, fatty and waxy, signify subacute (parenchymatous) nephritis; with large amount of leukocytes, suppurative nephritis.

In severe acute renal inflammations clusters of kidney epithelia, as well as cast-like masses of them, may be found.

EPIDERMAL SCALES.

These resemble epithelia but have no nucleus. They are flat, horny cells from the genitals, of pale, shriveled appearance, and may contain fat globules (smegma) and dust particles.

CHAPTER XXVIII.

TUBE-CASTS AND SIMILAR FORMATIONS. MASSES. CONNECTIVE TISSUE. SEMINAL FLUID. PROSTATIC BODIES. URETHRAL SHREDS.

Tube-casts: definition; constitution; difference in shape and constitution from cast-like formations.

Identification of casts: power to be used; appearance; distinction from extraneous objects,—epithelium, corpuscles, crystals, etc.; precaution in shading necessary.

Collection of the urine for identification of casts; criticism of nurses and others; causes of difficulty in finding casts.

Casts hidden by other constituents: phosphates, urates, pus; how to overcome this difficulty; staining the sediment; removal of blood.

Kinds of casts: true casts, crystalline, etc.

True renal casts: classification.

Hyaline casts: varieties; appearance; size; significance; relative proportion in renal lesions.

Waxy casts: appearance; color; amyloid reaction; size; fracture; significance; relation to prognosis.

Granular casts: varieties; composition; color; size.

Compound casts: significance.

Epithelial casts: composition; identincation; hyalo-epithelial casts; significance; occurrence in the renal lesions.

Fatty casts: composition; appearance; color; significance.

Blood casts: varieties; size; significance.

Leukocyte casts and pus casts: distinction; significance.

Bacterial casts: urate; oxalate; cystine.

Cast-like formations (pseudo-casts): appearance and composition; pseudo-casts of amorphous urates, phosphates, bacteria, etc.

Cylindroids (false casts): definition; appearance; significance; various authorities quoted; size and shape.

Mucous casts, threads, or ribbons: distinction from cylindroids; appearance and constitution.

Prostatic plugs: appearance, size, shape, etc.

Urethral shreds: "Tripper-Fäden," "clap threads," or gonorrheal shreds; appearance; composition; microscopical appearance; significance of different varieties; pus shreds; mucous shreds; mucous shreds; epithelial shreds; comma shreds.

Masses: classification and description; relation of fatty and granular masses to casts; bacteria, caseous, and fibrin masses.

Connective tissue: appearance of the fibers; distinction from mucous shreds and linen fibers; significance.

Diagnosis of masturbation in female children; role of connective tissue in bladder tumors.

Tissue fragments and portions of growths: demonstrations of elastic tissue-fragments and structureless masses from malignant growths.

Seminal fluid: spermatozoa; appearance; motion; size; other constituents of seminal fluid; significance of spermatozoa, "spermatorrhea," etc.; corpora amylacea.

Prostatic fluid and massage urine: definition of massage urine; composition; sago bodies, sugar granules, vesicular skins, vesicular casts, vesicular shreds; significance.

Tube casts are moulds of the uriniferous tubules and are formed of a protein exudate. The true nature of tube-casts has not been established, but they are probably composed of the coagulable elements of the blood, which, after gaining access to the renal tubules, entangle in them any free or partly detached products of the tubules and form moulds of them. "Dead epithelia are thus removed from the kidneys by transformation into a plastic permeable mass which is washed out by the urine in cast form."

Tube-casts proper consist of a uniform transparent gelatinous matrix to which other elements (epithelia, corpuscles, salt, etc.) may be accidently attached.

Cast-like formations have the same shape as casts, but lack the uniform matrix. They are composed of salts, corpuscles, and epithelia.

Cylindroids (false casts, mucous casts) are mucous molds with tapering ends.

Mucous threads (mucin threads) are narrower than casts or cylindroids and fade away imperceptibly.

Prostatic plugs resemble casts, but contain spermatozoa.

Urethral shreds are visible to the naked eye (so-called clap-threads or Tripper-Fäden) and occur as whitish cottony shreds which under the microscope may be very large, extending across the whole field.

IDENTIFICATION OF CASTS.

☞ In order to distinguish tube-casts from these formations as well as extraneous substances, hairs, fibers, etc., the following precautions must be observed; casts should be sought for with a low power and without cover-glass. The urine should be *acid.* Alkaline urine rapidly dissolves casts.

They may be recognized by use of a power of 150 diameters, when they will look small, yet much larger than corpuscles, spermatozoa, bacteria, or small crystals (as oxalate).

They are of *uniform breadth,* and usually longer than they are broad.

They have, usually, at least one well rounded extremity, and well defined borders.

They are not longitudinally striated, nor jagged, nor provided with processes; not jointed, segmented, nor serrated. They may possibly be spirally twisted at one or both ends.

They are distinguished from large epithelia by absence of the nucleus and by the well rounded extremity. They also refract differently.

They are distinguished from bacteria, corpuscles, spermatozoa and oxalate crystals by their larger size, uniform breadth, greater length than breadth, and rounded extremity.

They are distinguished from large crystals by absence of geometrical form and less refraction.

In some cases one end of the cast tapers off considerably, and presents a spirally twisted appearance, which may go on to such an extent that the entire cast becomes transversely striated. Broad hyaline casts may sometimes be branched dichotomously at one end.

☞ Their kind must be determined by use of a high power,—500 diameters.

☞ To find hyaline casts tilt the mirror of the microscope so as to darken the field gradually, when the outlines or shadows of delicate hyaline casts may be seen which otherwise might escape detection. Shading the mirror with a finger of the left hand serves the same purpose.

One of the author's assistants has humorously referred to a certain hospital interne as a man "who sometimes knows casts and sometimes not;" hence the necessity for precautions in collecting the urine properly and studying the sediment.

☞ **Collection of the Urine.**—Tube-casts are at times difficult to find in urine and certain precautions must be taken in regard to the collection of it. Freshly voided urine is to be preferred whenever it is possible to obtain it. Urine which is cloudy from bacteria or of unpleasant odor may be unfit for the search and a fresher sample should be demanded. Urine voided after the patient has been moved about is more likely to contain casts than that voided after repose. If urine to be examined for casts is to be sent from a distance, immediately add to four ounces of it five grains of boric acid and tightly stopper the container.

Patients and nurses usually furnish for examination the urine voided on rising in the morning. This is the poorest sample obtainable for the search for casts, unless perchance it happens to be the only sample which can be obtained fresh. Physicians should bear in mind this fact and assume that an unlabeled "bottle of urine" is urine voided on rising, unless proved to the contrary. One reason of the deplorable conflict in opinion as to the presence or absence of nephritis in a given case may be the fact that the "bottle of urine-doctor" has been unable to find casts in urine voided on rising in the morning.

Another cause of the difficulty in finding casts in hospital cases, even when the twenty-four hours' urine has by some happy accident been collected, lies in the fact that the day urine being older than the night has undergone bacterial decomposition in the foul hospital urinal which, so far as the writer knows, is nobody's business to clean. **Any vessel having an unpleasant odor is unfit for the reception of urine to be examined for casts.** Urine to be examined for tube-casts should, unless strictly fresh, be collected in sterile glass receptacles which are to be tightly closed and kept in a cool place. Nurses need repeated instructions in regard to this matter and even hospital internes, however scrupulously careful in regard to surgical infection, often forget the readiness with

which bacterial decomposition spoils urine for careful examination. Too many hospital reports read "casts negative" in cases in which proper collection would undoubtedly show presence of at least a few of these formations.

Again, after the urine has been collected, no matter how properly, delay or negligence in prompt examination may negative the findings, since in some cases the casts disappear quite rapidly. The author has frequently found casts in a sample of urine properly collected and promptly examined which have entirely disappeared at the end of 24 hours, being replaced by zoöglœa masses of bacteria. The rapid disappearance of casts has been attributed by some to urinary pepsin, by others to colon bacillus ferments.

In cases where there is difference of opinion as to the presence or absence of casts the freshly voided urine of every micturition during the 24 hours should be examined.

In cases where centrifuging for five minutes at a speed of from 1000-1700 per minute fails to reveal presence of casts boric acid (0.33 gramme per 120 c.c.) should be added to the urine, which is allowed to deposit spontaneously for six hours, the supernatant urine poured off and the sediment examined; if negative, the sediment should be centrifuged and examined.

☞ *Other constituents of the sediment* may be present in such mass as to hide casts. Phosphates, if present, should be dissolved by the addition of one drop of 50 per cent. acetic acid to the slide; amorphous urates should be dissolved by cautiously warming the urine to 50° C. (120° F.) which can be accomplished by setting the bottle for three minutes in water which is heated to 60° C. (140° F.). On no account should the urine be boiled. When pus is present in the urine, tube-casts can seldom be found. The best way to overcome the difficulty in this case is to *examine the urine first voided after washing out the bladder.* Women with vaginitis, leucorrhea, etc., should take precautions to exclude admixture of vaginal fluids with the urine, which can be done by douching or tamponing. In cases where it is proper, catheterization will furnish urine uncontaminated by vaginal

mucus, etc. In cases where much epithelial debris is present, confusing the observer by the number and variety of forms, *staining the sediment with Lugol's solution* should be resorted to. Three drops on a slide is sufficient to stain and to show the marked difference in form between epithelia and casts if present.

Blood when present in large amount may in some cases hide casts. In such cases centrifuge, pour off the supernatant urine, fill the tube with water, stir gently until the reddish sediment is dissolved and centrifuge again.

KINDS OF CASTS.

Casts may be divided into the following groups: true casts, crystalline casts, bacterial casts, and false casts.

True renal casts are hyaline, waxy, granular, epithelial, blood, leukocyte, and fatty.

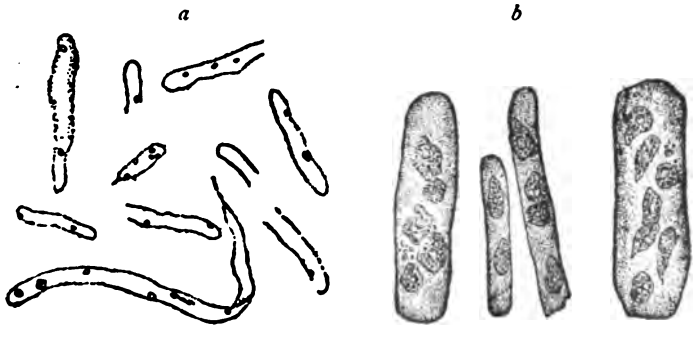


FIG. 49. (a) Hyaline, (b) Epithelial.

☞ *Hyaline casts* (Fig. 49) are of two varieties; pale and glassy.

Hyaline casts are transparent and homogeneous, generally with rounded ends. In size they are usually narrow and either short or long. When short, they may also be wide. Their sides are parallel and straight, as a rule; sometimes indented. They are either free from granules or else may contain a few fine pale granules, a drop or two of oil, or a renal cell or blood corpuscle. The narrow casts are supposed to be derived from the smaller

undenuded convoluted renal tubules, those of large diameter from the collecting tubules.

☞ According to Emerson it is clinically important to distinguish pale almost transparent hyaline casts from those of glassy appearance, the former occurring in circulatory disturbances of the kidney without nephritis, the latter a common form in some cases of nephritis.

Large hyaline casts, as a rule, indicate extensively denuded tubules, the result of advanced kidney lesions, those from the collecting tubules being the largest.

Hyaline casts which contain blood or renal epithelium are of pathological import and are held by some writers to indicate distinct inflammation of the kidney.

Hyaline casts occur (a) in irritation of the kidneys due to

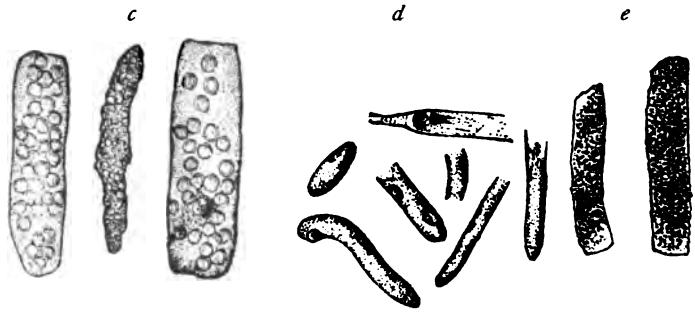


FIG. 50. (c) Blood casts, (d) finely granular, (e) coarsely granular.

dietetic errors, use of drugs, intestinal autointoxication, etc., (b) in congestions, (c) in nephritis. They are most numerous in acute processes, but are also found in chronic nephritis (interstitial) and in amyloid disease. In number they predominate in chronic interstitial nephritis (both primary and secondary), passive hyperemia and amyloid. The relative proportion of hyaline casts is smaller in comparison with other casts in acute and subacute lesions.

All hyaline casts are soluble in acetic acid. In breadth they range usually from 0.01 to 0.05 micromillimeter.

Some observers advise against use of the centrifuge in the

search for hyaline casts, claiming to find them more abundant when the urine spontaneously deposits them.

☞ *Waxy casts* (Fig. 51) differ from hyaline in being easy to see when found, since they are highly refractive, of sharp contour and homogeneous. They fracture easily and show a marked tendency to split transversely, hence sometimes are in very small pieces. They are broader, as a rule, than hyaline, and some are quite short; others may be large,—both long and broad. Their appearance suggests wax. In color they are yellowish like beeswax, or bluish white like paraffine. The term "fibrinous" was formerly applied to the yellowish casts. Some (not all) give the amyloid reaction with methyl-violet (red color), or with iodine solution (mahogany turning to dirty violet with 5 per cent. sulphuric acid). The iodine solution used for staining is made by dissolving 1 part of iodine in a solution of 10 parts potassium iodide in 100 of water.

Waxy casts may be dotted with crystals or granules, or have the same adhering elements as hyaline casts. They are but slowly soluble in acetic acid. They may be enormous in size, in some cases as large as 0.102 micromillimeters in diameter, but usually occur in short, stout fragments owing to brittleness. Occasionally a cast is seen split in twain, the two sections still held together by a narrow undivided portion.

Waxy casts probably represent a further chemical modification of the granular débris found in epithelium.

According to some writers the yellow, waxy or fibrinous casts signify acute renal processes and are of temporary occurrence. Following suppression of urine and oliguria waxy casts are found when the urine begins to increase. In a case of brain-tumor waxy casts were found by one observer.

☞ The persistent presence of waxy casts, however, is in the writer's experience one of the most reliable signs of serious renal disease, whether subacute or chronic. The author has not yet seen them persistently in abundance in strictly acute processes from which recovery took place.

Prognostically, therefore, the waxy casts persisting in urine are unfavorable, especially if of the "paraffine" variety (bluish-white).

☞ **Granular Casts.**—Granular casts (Fig. 50 d, e) consist of a hyaline matrix and well defined boundary with granular matter adhering to the matrix or imbedded in it. Granular casts may be finely or coarsely granular, yellow, brownish-red or almost black. Light-brown granular casts also occur, which are not so opaque as the brown-red (hemoglobin) casts.

The granules are thought to be the result of the degeneration and disintegration of the renal epithelium, but the yellow and brownish-red casts probably derive their color from destruction of red blood corpuscles and leukocytes. When bile is present in the urine, however, the yellow or brown color is due to bile pigment. Brownish-red granular casts are said to accompany blood casts frequently.

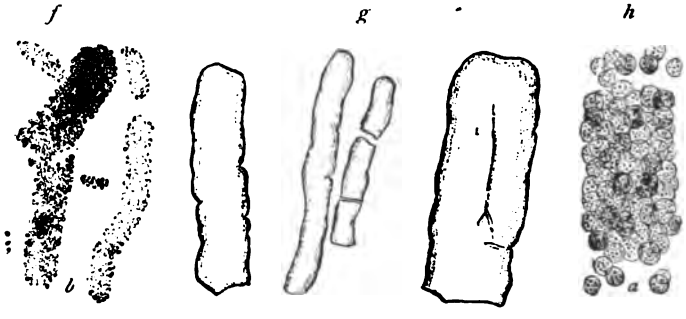


FIG. 51.—Tube Casts: *f*, urate cylindroids resembling granular casts; *g*, waxy; *h*, pus.
The narrower casts come from the narrow portion of the loop tubules; the broader from the straight collection tubules; the medium from the convoluted of the second order. Magnified 500 diameters.

Granular casts may be small or large, the large, long, straight, dark granular casts being derived, it is thought, from the collecting tubules. Granular casts are often broken off, leaving a concave or zigzag end instead of rounded. When the epithelia of casts is so degenerated as to be difficultly recognizable, the term granular is preferably used in designation. Compound casts occur, *i. e.*, granular-waxy.

☞ Granular casts indicate nephritis, except possibly yellow

granular ones, which may occur in cardiac cases, congestions, etc.

The author finds the large dark, coarsely granular casts in the more serious lesions, seldom in acute processes terminating in recovery. Granular casts also occur in degenerations.

☞ *Epithelial casts* (Fig. 49 b) are those which are made up of a hyaline matrix with cells of renal epithelium either imbedded or adherent, and either aggregations of cells which have scaled off and become massed or else, according to Emerson, scaled off portions of the tubules themselves. Round and vesicular nuclei must be determined in the cells in order to recognize the epithelial cast. The cells may be either well preserved or have undergone granular or fatty degeneration.

Epithelial casts with degenerated epithelia shade off into coarsely granular and fatty casts. *Identification of nucleated epithelium* determines the character of the cast. The hyaline matrix in epithelial casts is more or less concealed by the epithelia, but close observation will usually show a fine boundary line at some portion of the structure. A drop of acetic acid dissolves the matrix and sets free the epithelia.

In cases where hyaline casts have but few epithelia adherent to them the term *hyalo-epithelial* is used.

Leukocytes may be mixed with the epithelia in epithelial casts, but such casts are not true pus casts.

Well formed or perfect epithelial casts mean an acute process and by the number of them present the intensity of the process can be judged. They are significant of severe acute congestion, acute nephritis or of an acute exacerbation of a chronic nephritis. In severe acute congestions epithelial cylinders with a lumen and with intercellular protoplasmic bridges have been found.

☞ These epithelial casts are rare in chronic renal lesions and in amyloid, fairly common in subacute cases, especially during acute intercurrent attacks.

☞ *Fatty casts* (Fig. 52) have the hyaline matrix dotted with fat granules and droplets. The term is not used unless the cast is thickly coated with them. The fat globules may be small but refract highly, *i. e.*, glisten. When large, the oily appearance is

unmistakable. Fine needles of fatty acid crystals may project from the cast and fatty renal epithelia or granular fatty cells may adhere to them or be imbedded in them. Fatty casts are yellowish or may be almost black in color. They are soluble in ether. The fatty globules often preserve the outline of the original epithelia which have undergone fatty degeneration.

☞ Fatty masses and free fat accompany fatty casts. Fatty casts indicate extreme renal cell degeneration and are most common in subacute glomerular nephritis, especially in protracted cases. They also occur in secondary chronic interstitial nephritis, but, in the latter, more commonly fatty masses alone are found



FIG. 52.—Fatty Casts, Fatty Epithelia and Free Fat (after Bartley).

or with but few fatty casts. Fatty casts may, though not commonly, be found in the fatty stage of an acute nephritis and it is said occasionally in severe renal congestions.

It is difficult to reproduce fat and fatty casts in an illustration in such a way as to show the microscopical characteristics, but Bartley, in his excellent work on "Clinical Chemistry," has succeeded better than others with whom we are familiar. Bartley's representation is shown in Figure 52. His book on "Clinical Chemistry" should be studied in connection with this subject.

Blood Casts.—There are two varieties (a) the hyaline or granular casts covered with blood corpuscles and (b) the coagulated

fibrin cylinder with embedded blood corpuscles. In some cases the blood corpuscles are agglutinated and pale, hence difficult to recognize. Blood casts are not large and in one portion may be hyaline of granular (Fig. 50 c).

☞ Blood casts indicate hemorrhage into the kidneys. They are most common and abundant in acute post-scarlatinal nephritis, but may be found in any renal hematuria, as, *e. g.*, that of stone, cancer, congestion, tuberculosis, hemorrhagic infarcts.

If the blood corpuscles can be made out to be smaller and less colored than normal, the hemorrhage is either high up in the kidney or very slight. If of normal blood corpuscles either from the straight tubules or very abundant.

Leucocyte or Pus Casts.—The terms should not, perhaps, be used synonymously. Hyaline or granular casts with a few leukocytes adherent are frequently found in acute renal lesions and should be called leukocyte casts. On the other hand, casts completely covered with pus corpuscles (Fig. 51 h) are rarer and are found only in suppurative processes. Pus corpuscles differ from renal epithelium in having more than one nucleus, shown by adding acetic acid.

☞ True pus casts are found in chronic renal suppurations, *i. e.*, abscess, tuberculosis, pyelonephritis; large irregular plugs of coagulated material covered with pus are found in chronic inflammations of the renal pelvis with extension into the straight tubules.

☞ **Bacterial Casts.**—These rare formations are cylinders covered with bacteria and should not be confounded with bacteria masses common in stale urine. They are short, highly refractive and few in number, three or four usually being all found in the sediment of 10 c.c. of urine. They are insoluble in acetic acid.

Bacterial casts mean infected kidney and occur in urinary septicemia, most commonly in prostatics with ascending pyelonephritis (surgical kidney); hence recognition of them is of the utmost importance and a grave prognostic sign. They must be sought for in strictly fresh urine.

Crystalline Casts.—Casts occur in which masses of crystals can be seen either of ammonium urate (hedgehog), cystine, or oxalate. They signify deposit of crystals within the kidney.

FORMATIONS SIMILAR TO TUBE-CASTS.

A number of formations occur in the urine which are not true renal casts and whose significance is not the same as casts, but which bear more or less physical resemblance to them.

Cast-like Formations (pseudo-casts).—These are aggregations of various elements assuming the cast shape but lacking the matrix soluble in acetic acid.

☞ Amorphous urates and phosphates (Fig. 51, f) are the most common. A curious instance, however, of resemblance to casts, unrecorded in any book with which the author is familiar, is to be found in the tendency of pus corpuscles when abundant to roll together into cast-form on a slide over which a current of sediment is flowing in one direction or another. Pseudo-casts show on close inspection an irregular outline.

Amorphous phosphates in cast-form exactly simulate grayish granular casts in color, etc., but lack the outline and are wholly soluble in a drop of weak or strong acetic acid. Amorphous urates may occur simulating granular casts in form. They disappear when the slide is warmed.

Bacteria may be grouped in a cast-like manner, but on close inspection show irregular outline, and abundance of grouping not in cast form.

Hæmatoidin and granular detritus may also assume the cast form. Epithelia may be found in cast form. Such formations are hollow, being thrown off en masse from the uriniferous tubules. Seen only in parenchymatous nephritis, *i. e.*, Subacute.

Blood corpuscles enmeshed in fibrin are common in renal hemorrhages and may assume the cast form.

The differential diagnosis between a true hyaline cast and a cast-like formation can be made by addition of a drop of acetic acid, which dissolves the hyaline true cast, but has no effect on a cast-like formation.

☞ **False Casts or Cylindroids.**—This term should be restricted to formations exactly resembling the hyaline casts for most of their length, but which taper off at one end. Emerson says "it is perhaps safest to observe the old rule and exclude all from the

list of casts which have a definite tail." It is possible that if the tail is broken off in the centrifuge, the other portion can not be told from a true hyaline cast. If covered with urates, they resemble granular casts.

What is the significance of cylindroids? To this question we find the following answers in the books:

"They occur where casts would be expected practically always with true casts and have the same significance as they." (Emerson.)

"It is sufficient to say that they are apparently not true casts, that they are frequently present in a urine that is free from albumin, and that they are of little clinical importance." (Ogden.)

"Their true chemic nature is not exactly known, but they have little clinical significance, and are frequently present without albumin." (Saxe.)

"These formations may occur in normal or pathological urine and have no particular clinical significance." (Hawk.)

"They are not characteristic of kidney disease, but probably more often caused by irritation of the lower urinary tract which has in a measure extended to the kidneys." (Purdy.)

"They do not necessarily indicate disease and generally arise from irritation of the kidney extending from the ureters." (Tyson.)

"Similar in appearance and nearly equal in significance to hyaline casts." (E. C. Hill.)

"They are not, therefore, characteristic of kidney disease. They are observed most commonly in the urine of children which may or may not exhibit albumin in the absence of other symptoms." (Jaksch.)

"Cylindroids of distinctly tubular origin are pathological in the same sense as hyaline casts, * * * they may be covered with blood or kidney elements; then their pathological significance is assured." (Croftan.)

Croftan is also of the opinion that while neither cylindroids nor hyaline casts can be considered typical of anatomic renal lesions they certainly indicate some transitory functional disorder.

The writer has noticed cylindroids in great number in the urine of a certain man whenever the acidity was high,—40 degrees or more,—disappearing or growing less in number as the acidity decreased. Hyaline casts and albumin were absent, but occasionally sugar (dextrose) in small quantity (0.25 per cent. or less) would appear.

In cystitis and in urethritis cylindroids occur resembling tubular cylindroids. As to the constitution of cylindroids: Hawk thinks the basic substance of them is often the nucleo-protein of urine. They are described as smooth, long, flat, transparent structures, with a rather smaller diameter than casts, with perhaps forked or branching ends, but more commonly with tapering ends. They may be very large and band-like. True cylindroids are probably not fibrillated nor striated; these characteristics apply to mucous casts, which should be distinguished from cylindroids. True cylindroids should be soluble in acetic acid.

Mucous Casts, Threads or Ribbons.—These are sometimes called cylindroids, but in the author's opinion are very different in appearance. Mucous casts are distinctly flat, while cylindroids are like hyaline casts. Mucous casts are long, narrow, fibrillated, or striated and insoluble in acetic acid. Some authors make no distinction between cylindroids and mucous casts, but this author agrees with Emerson, who says it is common and right to divide them into two groups and that the mucous threads are flat ribbons of mucus which no one would confuse with hyaline casts.

The mucous threads may be found in the nubecula of normal urine and hence are common in any urine containing an abundance of mucus. When it comes to the question of cylindroids, mucous threads, and the like, Emerson, in his splendid book on *Clinical Diagnosis,* makes the clearest distinctions and his work should be in the hands of every one interested in this phase of the study. His illustration of the different forms of these materials is by far the best of any with which the author is familiar and is shown in Figure 53.

Prostatic Plugs.—These have been described as large colorless or yellow moulds of the prostatic ducts, of cylindrical shape with

rounded ends or irregular in shape with spermatozoa or prostatic epithelium imbedded in them. They are said by Ogden to be found most commonly in mild inflammatory processes of the vesical neck and prostate. Emerson calls them large cylindrical masses of mucus, but he also speaks of prostatic casts "which must be rare."

☞ **Urethral Shreds.**—In the late stage of acute specific urethritis and in gleet when the urethral secretion becomes mucous in character and collects in the longitudinal furrows of the mu-



FIG. 53.—*a*, Cylindroids, *i. e.*, bodies much resembling hyaline casts; *b*, mucous threads; *c*, a spiral structure of material resembling hyaline casts or mucous threads; *d*, a vegetable thread. × 400. (Emerson.)

cous membrane, mucoid shreds are formed, so-called Tripper-Fäden, gonorrheal or clap-threads, varying in size from a few millimeters to one centimeter long and of white or yellow color. They are visible to the naked eye and appear as small cottony whitish masses which sink to the bottom of the glass. They are most abundant usually in the urine first voided on rising in the morning. They consist of a basic mucous substance in which are embedded leukocytes and urethral epithelia.

Under the microscope clap-threads (Fig. 54) appear as large aggregations of leukocytes and epithelium, usually very wide, but sometimes of medium width and without the definite line of renal casts.

Description or even notice of these most common constituents of the urinary sediment is curiously lacking in most books on urine analysis.

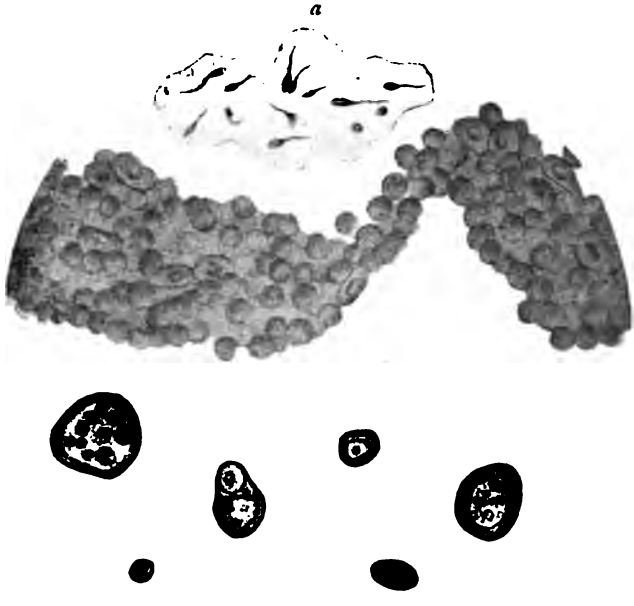


FIG. 54.—Corpora amylacea. *a*, Spermatozoa (Hofmann-Ultzmann); *b*, clap-thread (Hofmann-Ultzmann); *c*, corpora amylacea. Tubercle bacilli in urinary sediment.

☞ We are greatly indebted to Dr. G. A. De Santos Saxe, of New York, for extensive research work in clap-threads. According to Saxe urethral shreds appear with a fair degree of regularity with each stage of urethritis: pus shreds are the first signals of chronicity and appear in the chronic stage whenever an active inflammatory process is going on. As the urethritis becomes more chronic, the pus cells gradually disappear, the epithelium and mucoid matrix increase and the shreds come closer

to the mucopus variety, which signalizes a catarrhal type and a glandular involvement. The mucopus shreds are the most frequent and persistent; as exudation disappears and catarrhal condition is in evidence, mucous shreds appear, signalizing chronic soft infiltration with predominant glandular involvement. Single mucous shreds may persist for a long time. If the process now heals, even this last shred disappears, but if hard infiltration develops, shreds of flat epithelium are found.

Shreds, according to Saxe, should be fished out of the urine with sterile platinum wires and spread on slides. Here they are fixed with equal parts of alcohol and ether for 10 minutes, stained for from 1 to 2 minutes in Unna's polychrome methylene-blue, washed, dried, mounted in balsam, and examined. If detection of gonococci in shreds is desired, Gram's stain instead of the blue may be used. (See Gonococcus.)

Pus shreds are dense, heavy, opaque, yellowish white, often thick and shaggy, usually short and friable, sinking readily. Under the microscope we see innumerable pus corpuscles closely packed in a scarcely visible matrix of homogeneous character and containing urethral epithelium singly or in small groups. The urethral epithelium may have undergone hyaline degeneration, in which case Saxe has found them to stain diffusely and more reddish without chromatic distinction between nucleus and cell-body.

Mucopus shreds are longer than pus shreds, more wavy or twisted, very irregular in shape, thinner; more translucent, grayish-white and showing faint white linear streaks and occasionally large opaque nodes. One end may be curled into a knob. Microscopically there is more matrix, less pus, and more epithelium than in pus-shreds.

Mucus shreds are the lightest of all and float on or near the surface of the urine. They are long, thin, almost transparent, with faint greyish striations. Microscopically they consist of a mucoid matrix with a few epithelia and occasionally the remains of a few leukocytes. The matrix is arranged in fibrillated layers and stains deep purple.

Epithelial Shreds.—These occur in two varieties, either short, flaky, scaly masses loosely knit and shaggy, being characteristic of old chronic conditions, as in stricture, or less commonly very thin semi-transparent rapidly sinking bits which wrinkle and resemble desquamated epidermis.

Epithelial shreds are composed of flat (squamous) epithelia, large pale flat cells in pavement clinging to one another, occasionally overlapping. They are most common after passage of instruments or after application of silver nitrate.

Comma shreds are rare if true and are seldom found in urine except in the last glass of a five-glass test or after prostatic massage. They come from the prostatic ducts and outwardly resemble the comma-like mucopus shreds described above, but microscopically consist of epithelial masses from the prostatic ducts, usually in two layers of cells, one of cylindric and the other of small round epithelium (Saxe).

A number of elements found in the sediment remain to be considered, viz., masses, tissue fragments, portions of growths, organized blood clots, constituents of prostatic and seminal fluids, micro-organisms, parasites and worms.

Masses.—Substances in mass form composed of the same material as in casts, but without the characteristic cast shape, are frequently seen. *Fatty masses* (Fig. 52) are microscopically small, roundish, dark and highly refractive; they occur along with fatty casts and have the same significance as they, but are likely to persist in the urine of large white kidney after the fatty casts have disappeared and the patient fancies himself well.

Granular masses are also dark and resemble the fatty masses except that they are not so shiny. They occur along with granular casts and may be found when the latter have disappeared.

Both fatty and granular masses have distinct outlines like casts.

Bacteria masses are irregular in shape and outline, and dull in color and appearance. (See Zoöglœa.)

Caseous masses insoluble in acetic acid may occur in cases of tubercular ulceration of the urinary tract. These are microscopic.

Fibrin masses are found in the urine of chyluria, hematuria, tubercular kidney, etc. These are microscopic.

☞ **Connective Tissue.**—Little is to be found in medical literature regarding this substance in the urine except what has been written by Heitzmann. The appearance of connective tissue fibers in the urine is a frequent phenomenon. They are, as a rule, small and are distinguished from mucous threads by their greater refraction, their almost invariable occurrence in bundles of varying size, their fibrillary or finely granular appearance, and the presence in them at times of formations similar to nuclei. Linen fibers possess strong refraction but split in a way essentially different from connective tissue.

Shreds of connective tissue are found in the urine in:

1. Ulcerations.
2. Abscesses.
3. Tumors.
4. Hæmorrhages.
5. Trauma.
6. Cirrhosis and atrophy of the kidneys.
7. Hypertrophy of the prostate.

Connective tissue studded with fat is probably from the kidneys.

Connective tissue shreds indicate a disintegration of connective tissue, hence are signs as a rule of deep-seated and serious lesions. The diagnosis of masturbation when suspected in female children may be confirmed by the finding of connective tissue shreds in the urine, showing traumatism from rubbing.

In tumors of the bladder large shreds of connective tissue occur in rare cases in the urine, which can be found by dissolving the red sediment in water (after centrifugation) and again centrifuging.

These shreds appear under the microscope like branches of trees and sometimes almost like snakes, with a larger head-like part and a smaller tapering body.

Tissue Fragments, Portions of Growths.—Elastic tissue in large fragments may be demonstrated after centrifuging the urine, by dissolving phosphates in acetic acid, decanting the supernatant

urine and warming the sediment with a ten per cent. solution of potassium hydroxide, which destroys everything but the elastic tissue. The fluid is then sedimented again and examined microscopically.

Elastic fibers are recognized by their intense refractibility, wavy outline, sharp edges, uniform diameter, and curling ends.

Fragments of cancer and bladder growths may occur in size large enough to cut in sections. A structureless mass from a case of sarcoma of the kidney has been reported, 5.2 c.m. long and 0.5 c.m. wide, glassy, transparent and fairly firm (Emerson). As a rule inferences as to the nature of bits of tissue found floating in the urine cannot be made with certainty.

Seminal Fluid.—When mixed with the urine this fluid shows its constituents in the sediment.

☞ *Spermatozoa* occur in urine (Fig. 54) and are thread-like bodies consisting of an oval or pear-shaped head and a long, delicate tail, curling easily, like a whip. In urine less strictly fresh they are motionless. Their motion in fresh semen is eel-like and active. They can be seen plainly with 150 diameters, but for certain identification a higher power,—450 or more,—should be used. Their entire length is about 1-600 of an inch. In urine the spermatozoa are often accompanied by coarsely granular and finely granular epithelium; occasionally by lecithine corpuscles and corpora amylacea. Spermatozoa occurring occasionally in the night urine of men is of no significance except that of emission or coitus. In women the urine may contain spermatozoa in small number many hours after coitus, even though several micturitions have taken place in the meantime. Pathologically spermatozoa are found in the urine of men at times in acute fevers (typhoid, sepsis, pneumonia) and after epileptic fits, convulsions, etc. In cases of severe constipation the irritation of hard scybala upon the seminal vesicles may cause partial evacuation of semen. Constant presence of spermatozoa in the urine suggests spermatorrhea, as in venereal excesses and masturbation, or it may be referable to acute or subacute prostatitis, vesiculitis, or prostatic irritation and congestion. Spermatorrhea may also be noticed in certain spinal diseases. A tight stricture with

urethral dilatation behind the constriction may cause spermatorrhea by irritation from alkaline urine. Inflammatory conditions present are suggested by presence of pus in addition to spermatozoa, and in rare cases by finding the heads of the spermatozoa changed into pus corpuscles.

Corpora Amylacea.—These bodies (Fig. 54) appear like starch granules, but are colored red instead of blue by methyl-violet. They are found in the acini of the prostate and from there get into the urine, in the sediment of which they are seen by the microscope as opaque spheroidal bodies, either homogeneous or lamellated and containing within them a darker core, sometimes densely lamellated, sometimes colored. In old men they may form the nuclei of prostatic concretions. Corpora amylacea have no significance in urine.

Boettcher's crystals, spermin crystals, though present in prostatic secretion, are said never to be found in acid solutions, hence to be absent from the urine.

Prostatic Fluid and Massage Urine.—By massage urine is meant that voided by the patient after massage of the prostate and vesicles by the finger of the operator in the rectum. Such urine has been thoroughly studied by Saxe, who finds in it "sago bodies, sugar granules, vesicular skins, vesicular casts and vesicular threads."

"*Sago bodies*" come from the recesses of the seminal vesicles and are masses of colloid material of the size of a small pea, in which are embedded numerous motionless spermatozoa with epithelium from the vesicles and here and there a leukocyte.

"Sugar granules" are much smaller, about the size of a pin head; are glassy and translucent, settle and dissolve rapidly. They are essentially the same microscopically as sago except that they contain fewer spermatozoa.

"*Vesicular skins*" are fine, almost translucent, whitish pellicles, like the thin shells of lemon seeds, grouped in masses. Removed from the urine they collapse into viscid shreds. Microscopically they are the same as sago, but their elements are more densely packed and mixed with ropy mucoid.

"*Vesicular casts*" are moulds of the vesicles grouped into grape-

like masses or occurring in elongated sausage-like forms. They are semi-opaque, whitish and readily identified by the naked eye. Microscopically they are characteristic, showing a lobulated border with concentric layers of spermatozoa in dense masses on the edge and numerous spermatozoa in the central portions. They may also contain pus bacteria and gonococci.

"Vesicular shreds" are large and look like pieces of egg membrane. They present a matrix of mucoid in which are imbedded spermatozoa, epithelium from the vesicles, leukocytes and often bacteria.

Clinically, Saxe finds only the casts and shreds evidences of vesiculitis and especially when they contain leukocytes and bacteria. It is essential to fix the bodies on a slide (after washing with normal salt) with equal parts of alcohol and ether for fifteen minutes, to stain with eosin and then with methylene blue, as in the case of urine sediments previously described. Too much praise can hardly be given Dr. Saxe for his painstaking study of these bodies neglected by other authors. His book on the "Urine" should be owned by every one interested in genito-urinary work.

CHAPTER XXIX.

MICRO-ORGANISMS: MOULDS, YEASTS AND BACTERIA; PARASITES AND WORMS; EXTRANEOUS OBJECTS.

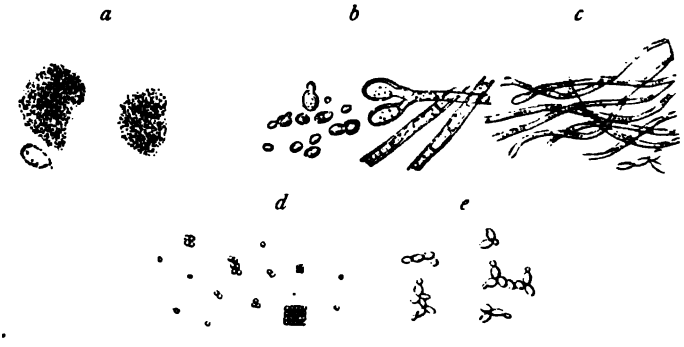
Moulds: penicillium, yeast fungus; sarcinae.
Importance of identification of spores.
Bacteria: classification: non-pathogenic germs; micrococcus ureae; urease; bacillus ureae, etc.
Bacteriological examination of urine: technique.
Pathogenic bacteria: classification: the bacillus coli communis; the bacillus typhosus; the bacillus tuberculosis; appearance.
Culture growth and identification of these germs; animal inoculation.
The bacillus of glanders; of malignant pustule; the bacillus pyocyaneus.
Pathogenic micrococci; "pus microbes;" streptococcus pyogenes and staphylococcus; appearance; identification; occurrence in diseases; the gonococcus of Neisser; identification in urine; differentiation from other organisms; relation to diseases.
Actinomyces; léptothrix; lactic acid bacillus.
Parasites and worms: classification.
Filaria sanguinis hominis: relation to chyluria; appearance of the embryo; pathology; hematuria; hematochyluria and chyluria.
Distoma hematobium: appearance of the worm; diseases caused by it and the eggs.
Echinococcus (hydatids): paratitic cysts of the kidney and hooklets in the urine; appearance of the worm and of the eggs; development and contents of the cysts.
Eustrongylus visceralis: appearance of the worm; action on the kidney.
Oxyuris vermicularis: appearance, etc.
Anguillula aceti: the vinegar worm and its appearance.
Extraneous objects: appearance under the microscope of fibers; cotton, linen, wool, and silk; feathers; hairs; moth wings; algae; cork; rust particles; air bubbles; oil globules; starch; lycopodium; cellulose.
Feces in urine: appearance and significance.
Flaws in the glass slide; dust, etc.

Micro-organisms.—These occurring in urine are either moulds,

yeast or bacteria. The urine in the bladder is in all probability sterile, but becomes infected, perhaps in the urethra, and certainly soon after it is voided, since, as Croftan aptly puts it, "it is a splendid culture medium for many varieties of air-borne micro-organisms."

Moulds are found, possibly in some cases when the urine is freshly voided, if catheters or instruments have been previously introduced. As a rule, however, the spores of moulds accumulate as the urine stands exposed to the air.

☞ The most common mould in urine is *penicillium glaucum,* occurring especially in acid urine. It is surprising how little space is devoted by the books to warning the beginner about this organism, which is so frequently seen in the urine and which



FIGS. 55 & 56.—*a*, Micrococcus ureæ in zöoglœa masses; *b* and *c*, penicillium; *d*, sarcinæ; *e*, yeast spores.

gives the tyro so much trouble. The spores closely resemble blood corpuscles, but refract higher and after a time multiply by linear division, forming threads of characteristic appearance.

☞ To distinguish penicillium spores (Figs. 55 and 56) from blood corpuscles the easiest way is to discover that some at least of the spores are oval and inclined to groups or clusters. Moreover the spores always have a cell body, *i. e.*, never appear as a ring and are always colorless.

The mycelium of penicillium may be present either as a single branch or in numerous branches, long, slender and serrated or

jointed. In some urines this mould grows luxuriantly and may occupy the entire microscopic field.

Its clinical significance is merely that of acid urine, but it may be mistaken for tube casts. Its branching appearance, narrowness and segmented structure should serve to distinguish it. The yeast fungus (saccharomyces urinæ) is identical with saccharomyces cerevisiæ and occurs for the most part in diabetic urine, in which so much may be present as to form a floating mass in or near the surface. This fungus may also be present in urine which has been placed in a container in which a little syrup or other saccharine substance remains.

The spores resemble those of penicillium. The sediment of old diabetic urine obtained by centrifuging may be composed of millions of the spores. (Fig. 56 e.)

There is no clinical significance to these bodies except the inference as to sugar-content of the urine.

Sarcinae have been occasionally found by the author in urine (Fig. 56 d). They resemble the lung sarcinæ occurring in cubes of eight cells, each cube forming a "bale of goods" as it were. No clinical significance has been established.

Bacteria found in urine are either non-pathogenic or pathogenic. When urine stands exposed to air it soon becomes turbid from the presence and activity of non-pathogenic bacteria. A considerable number of these are said to take part in the decomposition of urine, of which the *micrococcus ureæ* is thought to be the most important. Cloudiness due to bacteria is not removed by ordinary sedimentation nor by filtration. A high speed of the centrifuge may be required (5,000 revolutions) and unusual care in filtration.

☞ In stale urines the scum so often seen on the surface is due to micro-organisms, and in any case where they are abundant the urine presents a wave-like appearance or shimmer when shaken. Of the twenty or thirty germs which may produce alkaline fermentation of urea the micrococcus ureæ, which is constantly present in the air, is the most common, most abundant, and most important. It appears in a variety of forms in the

urine, (1) as chains of roundish short rods or highly refractory dots, (2) as little spheroids arranged in rows, or, (3) as zoöglœa masses of fine shiny spheres (Fig. 55 a). It changes urea into ammonium carbonate by the aid of a soluble ferment which it secretes, known as *urease*.

Other commonly occurring non-pathogenic organisms are the *bacillus ureæ*, the *staphylococcus ureæ liquefaciens*, the *uro-bacillus liquefaciens septicus* of Kragius, the *uro-bacillus Freudenreichii*, the *bacillus fluorescens*, the *staphylococcus ureæ candidus* and the *uro-bacillus Maddoxii*. These, like the micrococcus ureæ, have the power of producing alkaline fermentation of urea. It is claimed that the uro-bacillus Maddoxii is the organism capable of making the sediment viscid and stringy.

None of the non-pathogenic organisms possess clinical importance, unless they gain access to the bladder and produce abnormal changes in the urine in that organ. The non-pathogenic bacillus most common in urine is the *uro-bacillus liquefaciens septicus*.

☞ **Bacteriological Examination of Urine.**—The urine freshly voided into sterilized glass vessels should be supplied and long centrifuging,—five or ten minutes,—employed by the use of the author's special centrifugal tubes or better still in the small bacterial tubes of Purdy. These permit the pouring off of nearly all the supernatant urine, much less being left without disturbance of the sediment than in the case of the ordinary tubes. The sediment which remains may then be washed by repeated centrifuging with water and scraped off from the bottom finally with a sterile metallic loop, as that of platinum. In this way admixture with urea and other salts is reduced to a minimum. Urines which contain almost no sediment must be diluted with from 1 to 2 parts alcohol, allowed to settle spontaneously, decanted, and the sediment thus obtained concentrated by centrifuging in the author's special tubes. When the sediment is not too plenty, addition of alcohol before centrifuging helps. A little white of egg and glycerin may be added to the smear to make it adhere better to the glass. Cover the film with equal

parts of absolute alcohol and ether, let dry or pass through the Bunsen flame three times. Then the staining may be performed.

Dr. Purdy used special small centrifugal tubes and a speed of 5,000 revolutions for sedimenting bacteria in urine.

Pathogenic bacteria found in urine are of two forms, *bacilli* and *cocci*. Two common forms of bacilli are the *bacillus coli communis* and the *bacillus typhosus* of Eberth. The *bacillus tuberculosis* is found in some cases. The *bacillus pyocyaneus* also occurs.

☞ The **bacillus coli,** which really is a group of germs, is often found in acid urine and especially in cases of pyuria. It is derived from the intestinal tract, in which it occurs normally as a harmless saprophyte. In most cases it is thought that the colon bacillus is not the cause of the pyelitis and cystitis, though it is often found in these cases. On the other hand, it is held that in a minority of cases it is the causative agent primarily. Melchior found it in 37 out of 72 cases of cystitis; 29 times in pure culture. He claims it is non-pathogenic when injected into the healthy bladder, pathogenic when there has been irritation, obstruction, or injury previously. Pure cultures of this germ are now prepared for the treatment of urinary infections by the vaccine methods, and much credit is due to Dr. Edward C. Streeter, of Boston, for work along this line.

In thirty cases of urinary infection, examined by Drs. Hartwell and E. C. Streeter, the colon bacillus was the infecting organism in twenty-two. Mixed with pus organisms in urine, the streptococcus was the cause in one. That and the staphylococcus albus in two, and the staphylococcus albus alone in one. The use of autogenous vaccines was followed by an improvement of the symptoms in more than half the cases, but little effect was noted on the bacterium which undoubtedly renders the patient liable to recurrence of symptoms

According to Delafield and Prudden it is not possible to indicate very definitely all the conditions under which the colon bacilli are ordinarily harmless; intestinal saprophytes may gain access to the tissues and become actively pathogenic. Whether it is

special strains of the bacillus coming in from without which are pathogenic or whether the ordinary forms assume virulent capacities under unknown conditions we cannot tell today.

In shape the bacillus coli is a rod with rounded ends, sometimes so short, according to Abbott, as to appear almost spherical, while again it is seen as very much longer threads. Often both forms are associated in the same culture. It may occur as single cells, or as in pairs joined end to end. Its size is from 1 to 2.5 microns long and 0.5 micron thick.

It has no peculiar morphological features that aid in identification of it. It is usually said to be motile, and undoubtedly is motile in the majority of cases; but its movements are at times so sluggish that a positive opinion is often difficult.

It stains with the ordinary aniline dyes. It is decolorized when treated by the method of Gram (Abbott).

☞ The Eberth bacillus, **bacillus typhosus,** is the germ which may render the freshly voided urine of typhoid cloudy. It is said to be present in enormous numbers, even with hardly any pus, in about one-third the cases. According to some authorities, however, it is seldom found unless the urine also contains albumin. It occurs in abundance in the urine of typhoid cystitis. Differentiation of the typhoid bacillus from the colon bacillus requires care and use of culture. According to Abbott the typhoid bacillus is about three times as long as it is broad, with rounded ends. It is about one-third as long as the diameter of a red blood cell. It may appear at one time as very short ovals, at another time as long threads, and both forms may occur together. Its breadth remains tolerably constant. It is aërobic, facultative anaerobic and asporogenous.

The morphology of it presents little that will aid in its identification. It is actively motile and when stained by special methods is seen to possess very delicate locomotive organs in the form of very fine hair-like flagella, attached in large numbers to all parts of its surface. These flagella are not seen in unstained preparations, nor are they rendered visible by ordinary methods of staining.

Owing to a tendency to retraction of its protoplasm from the

cell envelope and the consequent production of vacuoles in the bacilli, the staining of this organism is frequently more or less irregular. At some points in a single cell marked differences in the intensity of the staining will be seen, and here and there areas quite free from color can commonly be detected. These colorless portions are often so sharply defined that they look as if they had been punched out with a sharp instrument. It does not form spores.

By comparing the *bacillus typhosus* and *bacillus coli,* it will be seen that, while they simulate each other in certain respects, they nevertheless possess individual characteristics by which they may be readily differentiated. The least variable of the differential points are:

1. Motility of *bacillus typhosus* is much more conspicuous, as a rule, than that of *bacillus coli.*

2. On gelatin, colonies of the typhoid bacillus develop more slowly than do those of the colon bacillus.

3. On potato, the growth of the typhoid bacillus is usually invisible (though not always); while that of the colon bacillus is rapid, luxuriant and always visible.

4. The typhoid bacillus does not cause coagulation of milk with acid reaction. The colon bacillus does this in from thirty-six to forty-eight hours in the incubator.

5. The typhoid bacillus never causes fermentation, with liberation of gas, in media containing glucose, lactose or saccharose. The colon bacillus is conspicuous for its power of causing gaseous fermentation in such solutions.

6. In nutrient agar-agar or gelatine containing lactose and litmus tincture, and of slightly alkaline reaction, the color of the colonies of typhoid bacillus is pale blue, and there is no reddening of the surrounding medium; while the colonies of the colon bacillus are pink and the medium round them becomes red.

7. The typhoid bacillus does not, *as a rule,* possess the property of producing indol in solutions of peptone; the growth of the colon bacillus in these solutions is accompanied by the production of indol in from forty-eight to seventy-two hours at 37° to 38° C. (98°.6 to 100°.4) (Abbott).

☞ The **bacillus tuberculosis** is occasionally found in urine, although in many cases of undoubted urinary tuberculosis it is difficult or even impossible to find it.

Microscopically, the organism itself is a delicate rod, usually somewhat beaded in its structure, though rarely it is seen to be homogeneous. It is either quite straight or somewhat curved or bent on its axis. In some preparations involution forms, consisting of rods a little clubbed at one extremity or slightly bulging at different points, may be detected. Branching forms of this organism have been described. It varies in length—sometimes being seen in very short segments, again much longer, though never as long threads. Usually its length varies from 2 to 5 microns. It is commonly described as being in length about one-fourth to one-half the diameter of a red blood corpuscle. It is very slender.

These rods usually present, as has been said, an appearance of alternate stained and colorless portions. The latter portions are believed to be the spores of the organism, though as yet no incontestable proof of this opinion has been presented. At times these colorless portions are seen to bulge slightly beyond the contour of the rod, and in this way give to the rods the beaded appearance so commonly ascribed to them.

Cultures of the bacillus tuberculosis are characteristic, dry masses in flat scales or coarse granular masses, like dry meal, adhering so tenaciously together that it is with the greatest difficulty that they can be separated. Grinding in a mortar with a foreign substance is necessary in order to pulverize them. The cultures on serum or glycerin agar are dirty drab or brownish gray. On potato they are nearly the same color as the potato and on milk-agar they resemble the color of the medium.

In bouillon they grow as a thin pellicle on the surface. This may fall to the bottom of the fluid and continue to develop, its place on the surface being taken by a second pellicle.

The tubercle bacillus does not develop on gelatin because of the low temperature at which this medium must be used.

A peculiarity of this organism is its behavior towards staining reagents, and by this means alone it may be easily recognized.

The bacillus does not stain by ordinary methods and accessory agents are necessary to intensify the stains or render the protoplasm of the bacilli more accessible to them.

☞ For *detection* of tubercle bacillus in urine the latter should be freshly voided and preferably by use of the catheter after washing or douching the external genitals. In stale urine decomposition may have destroyed the organism and in urine voided naturally the smegma bacillus from the external genitals may be a source of confusion.

The sediment is obtained as described above. (See Bacteriological Examination of Urine.)

☞ Instead of waiting for the smears to dry they may be fixed for staining by passing through the Bunsen flame three times. Several slides should be prepared for use. After fixing the smear it should be immersed in Ziehl's solution, made by adding to a five per cent. aqueous solution of carbolic acid about one-tenth its volume of saturated alcoholic solution of fuchsin. The slides should be immersed in the Ziehl solution in a dish or tray which can be heated over the Bunsen flame until the dye boils well. Then let the hot slides stand two minutes, after which they are removed from the flame. Excess of dye is allowed to drip off and the slide blotted. The decolorizer is then applied to remove the stain from all constituents except the bacilli. Various agents have been suggested, as a five per cent. solution of sulphuric acid in water or alcohol; according to Saxe the best one is a three per cent. solution of pure hydrochloric acid in 95 per cent. alcohol; Abbott advises 30 per cent. aqueous nitric acid. Nuttall for decolorizing prefers, after staining, to pass through three alcohols and to wash in a solution of 20 to 30 drops of pure sulphuric acid in 150 c.c. of water and 50 c.c. of alcohol. ☞ W. Henry Wilson, of Chicago, prefers two per cent. pure hydrochloric acid in 80 per cent. alcohol.

Ogden insists upon a second decolorizing with alcohol after use of the acid. His method is to decolorize in 20 per cent. aqueous nitric acid, wash in water and finally still further decolorize for at least ten minutes in 70 per cent. alcohol, as the smegma bacillus is decolorized by alcohol quite readily. ☞ W.

Henry Wilson does not find this second treatment with alcohol necessary when two per cent. hydrochloric acid in 80 per cent. alcohol is used. To exclude the smegma bacillus Emerson thinks cleanliness the best preventive and claims that this bacillus varies in morphology and in staining characteristics. Some forms are exactly like the tubercle bacillus. Emerson, like Wilson, advises for decolorizing two per cent. hydrochloric acid in 80 per cent. alcohol. When well decolorized only the thickest parts of the smear show any red. The decolorizer is used by pouring it repeatedly on the slide until no more red color comes off and until the film appears grayish. The slide is then washed with water and the film counterstained with Loeffler's aqueous methylene blue for about two minutes, thoroughly washed in water, dried, mounted in balsam and examined with a 1/12 oil immersion lens. Plate II., after v. Jaksch, shows the tubercle bacilli in the urinary sediment.

For the methylene blue counterstain Emerson recommends Loeffler's, which, according to Abbott, is made by mixing 30 c.c. of a saturated alcoholic solution of methylene blue in 100 c.c. of a 1 to 10,000 solution of potassium hydroxide.

☞ According to W. Henry Wilson, of Chicago, the best methylene stain is that from a one per cent. aqueous solution of **rectified** methylene blue, Gruebler's color being used. One advantage of this solution is that it keeps indefinitely.

In fixing, too high heat should be avoided, since it injures the staining quality of the bacilli.

☞ Finally, it must be carefully borne in mind that failure to find the bacillus tuberculosis by no means excludes presence of the disease. Repeated examinations of freshly voided urine may be necessary and even then the organism may escape detection.

☞ **Pappenheim's Method.**—Stain in carbol-fuchsin by steaming near the boiling point for three or four minutes; pour off the excess of carbol-fuchsin and treat without washing with Pappenheim's solution, pouring it slowly three or four times over the preparation, and allow it to drain off; wash in water, dry and mount.

Pappenheim's solution consists of corallin, one part dissolved



PLATE II.

Tubercle Bacilli in Urinary Sediment

(After v. Jaksch.)

in 100 parts of absolute alcohol, to which sufficient methylene blue in bulk is added to saturate. Finally 20 parts of glycerine are added and the whole thoroughly mixed.

Laboratory Note.—A special precaution necessary in examining urine for tubercle bacilli is to get fresh urine and centrifugalize a great quantity of it, softening the pus, according to Biedert's method, with as little 10 per cent. sodium hydroxide solution as possible. In order to make the bacilli stick to the slide the smear may be mixed with a little blood serum or egg albumin.

Animal Inoculation.—Great precautions are necessary to prevent infection with pus microbes and death of the animal from this cause. The centrifugal tubes should be cleaned out with the sodium hypobromite solution used for analysis of urea, then washed in alcohol and in boiling water repeatedly until sterile. The urine is centrifuged in these tubes and after decantation is again centrifuged several times with addition of sterile normal salt solution and decantation. In this way the urinary salts and other solids in solution are removed. The suspended sediment is then drawn up into a sterile hypodermic syringe and injected in amount 1-2 c.c. into the shaved and aseptic groin of a guinea pig as nearly as possible in the region of the glands. The region is massaged and the glands, if felt, are squeezed for a minute or two. The animal is then well cared for during a period of six weeks, after which it is chloroformed to death and a post-mortem held for determining the presence of tuberculosis in the lungs or elsewhere. If the inguinal glands swell the animal should be killed in three weeks and sections of these glands made. Animal inoculations are seldom practiced except for didactic work.

The bacillus of glanders, *bacterium Mallei, bacillus Mallei,* occurs in urine. It is a slender bacillus proportionately thicker than the bacillus tuberculosis, occurring singly or in pairs. It stains easily with the aniline dyes, but is readily decolorized by dilute acids or dilute alcohol. It is left decolorized by Gram's method.

The bacillus of malignant pustule (anthrax bacillus), *bacillus anthracis,* occurs in urine and is from 5 to 20 microns long and about one micron broad, capsulated and often uneven along the

sides. The ends of the bacilli are square or slightly concave and the bacilli often hang together end-to-end, forming thread-like structures. They are immobile, sometimes capsulated and easily stained by aniline dyes. They grow readily on artificial culture media at ordinary room temperatures, fluidifying gelatine, and usually growing out, before they do so, in a network of delicate filaments into the solid medium. If cultures of the anthrax bacillus be made at a temperature of about 42° C., growth occurs but is meagre. Spores are not formed as at body temperature and the virulence of the germ diminishes day by day.

The *bacillus pyocyaneus* (pseudomonas æruginosa) is found in urine. The name is given to it since it can impart a greenish color to pus. It produces most obstinate suppurations and may remain in the urine after all other germs have been removed.

Appearance of it alone, therefore, usually indicates an old infection. It forms a delicate rod with rounded or pointed ends, is actively motile, does not form spores, and prepared from cultures is commonly clustered in irregular masses. Most frequently it occurs in single cells, seldom more than four being joined end-to-end. It grows readily on all artificial media and gives them a bright green color. It is an active producer of a proteolytic enzyme. It stains with ordinary dyes.

Micrococci in urine of pathogenic importance are the streptococcus pyogenes, the staphylococcus pyogenes albus, citreus, and aureus; the gonococcus of Neisser is also an important coccus. The streptococcus and staphylococcus are collectively known as "pus-microbes."

☞ **Staphylococcus pyogenes** occurs as aureus, albus, and citreus. The aureus is a relatively small coccus, 0.7 to 1.2 microns in diameter. It grows in irregular masses and heaps, sometimes in pairs or groups of four, or in short rows. It is readily stained by anilins and is not decolorized by Gram's method. It does not show spontaneous movement nor develop spores. It forms somewhat voluminous masses of culture in the ordinary culture media. It rapidly fluidifies gelatin through formation of a ferment, coagulates milk and in the various media develops a yellowish white or deep golden yellow color, whence the name, aureus. Liability

to suppuration is greatly increased by mechanical or chemical injury to the tissue with which the germ is brought into contact.

"It incites these changes in the body by virtue of certain toxins or toxalbumins which are produced as the result of its metabolism, and which are either at once set free or stored up in the body of the germs as endotoxins until their release by disintegration after the death of the germs." (Delafield and Prudden.) It is thought that amyloid disease of the kidneys is due to the action of the staphylococcus following long-continued suppurative process.

Staphylococcus albus resembles the aureus, but does not develop the yellow color in cultures.

Staphylococci are particularly common in ammoniacal urine and can produce ammoniacal decomposition of urine. If the only germ found in freshly voided purulent urine, the infection is likely to be obstinate.

Streptococci are not so common in the urine as staphylococci, but are found in some forms of nephritis and cystitis. They also occur in the urine of erysipelas and in streptococcus pyemia with nephritis.

☞ **Streptococcus pyogenes** is the organism most commonly found in rapidly spreading suppurations. It exhibits a marked tendency to hang together in *chains* both long and short. It grows readily on the ordinary culture media, and does not fluidify gelatin. It develops small grayish colonies on agar kept at 37° C. for 24 hours, which, under the microscope, show loops and fringes of the chain-like cocci. In nutrient broth it usually forms delicate flocculent masses clinging to the sides or diffused. When in vigorous growth it coagulates milk and develops hemolytic substances in its growth. Both the streptococcus pyogenes and the staphylococcus aureus are what are known as Gram-positive germs, that is, are stained purple by gentian-violet and iodo-potassium iodide solution, which color is not removed by alcohol. (Abbott.)

☞ **Gonococcus** (micrococcus gonorrhœæ). Occasionally

as when in chronic gonorrhea there is but little discharge, it may be necessary to examine the urine for the gonococcus of Neisser. Ordinarily the gonorrheal discharge from the urethra is examined directly. It is difficult to find the gonococcus in urine and the best for the purpose is that voided after massage of the prostate and vesicles; or the urine is voided into two glasses and that containing the most sediment is centrifuged, the thickest part of the sediment spread on several slides and allowed to dry spontaneously. It is then fixed according to the alcohol-ether method or by the heat method and stained by Gram's method.

The gonococcus is an intracellular coccus readily and intensely stained by the simple anilin dyes, but does not stain purple with the Gram method. It is a diplococcus likely to ocur in pairs of kidney shape, with flattened surfaces facing each other.

The gonococcus does not grow at ordinary room temperature nor on the ordinary solid or fluid culture media. It may, however, be cultivated at the temperature of the body on human blood-serum or on a combination of this with agar.

Certain pathological fluids from the human body, as ascites fluid, ovarian cyst fluid, pleuritic serous effusions and fluid from joint cavities have been used for culture media. Lipschuetz prepares a culture medium from Merck's pulverized egg-albumin as follows: a 2 per cent. solution of the egg-albumin is made in water to which is added 20 c.c. of a decinormal caustic soda solution per 100 c.c. of fluid. The mixture is allowed to stand 30 minutes with occasional shaking. It is then filtered and placed in Erlenmeyer flasks in quantities varying from 30 to 50 c.c. and sterilized by the intermittent method. Thus prepared the medium is colorless, transparent, of light-yellow color and alkaline to litmus paper. To it is added nutrient agar, or the ordinary bouillon may be added in the proportion of one part of the egg-albumin medium to two or three parts of the agar medium or the bouillon, and this he calls the "egg-albumin-agar" or the "egg-albumin-bouillon" medium, on which micrococcus gonorrhœæ grows very satisfactorily. The special advantages claimed for this medium are that it can be prepared at any time and without

difficulty, is quite clear and transparent, and permits, where agar-agar is used, the employment of the medium for the study of colony formations. (Abbott.)

Since gonorrhœal discharges may be contaminated with pyogenic cocci other than those causing the specific inflammation, it is important in efforts to isolate this organism that the differential tests be borne in mind and put into practice. The gonococcus is differentiated from the commoner pyogenic organisms by the following peculiarities:

☞ First, it is practically always seen in the form of diplococci, the pair of individual cells having the appearance of two hemispheres, with the diameters opposed, and separated from one another by a narrow colorless slit.

Second, in gonorrheal pus it is practically always to be found *within* the protoplasmic bodies of the pus-cells.

Third, it stains readily with the ordinary staining reagents, *but loses its color when treated by the method of Gram.* In other words, the gonococcus is Gram-negative.

Fourth, it does not develop upon any of the ordinary media used in the laboratory; while the common pus-organisms, with perhaps the exception of the streptococci, are vigorous growers and are not markedly fastidious as to their nutritive medium.

Fifth, when obtained in pure culture by either of the special procedures noted above, its cultivation may be continued upon the same medium; but growth will usually not be observed if it is transplanted to ordinary nutrient gelatin agar-agar, bouillon, or potato; should it grow under these circumstances development of it will be very feeble.

Sixth, it has no pathogenic properties for animals. (Abbott.)

The gonococcus not only causes gonorrhœa, but also gonorrhœal prostatitis and vesiculitis, proctitis, Bartholinitis, and vaginitis. Gonorrhœal inflammations of the cervix uteri and even of the pelvis of the kidney also are known.

☞ **Detection of the Gonococcus.**—The sediment obtained and the film fixed as above described, it is stained for one and one-half minutes in a solution made by mixing one part of a saturated

alcoholic gentian solution with nine parts of a 5 per cent. carbolic acid solution. The slide is not washed, but excess of violet is merely poured off and the slide covered with the Gram iodine solution made by dissolving two parts by weight of potassium iodide in 300 of water, and in this solution one part of iodine dissolved. After a few seconds the iodine solution is poured off and a fresh amount poured on and this operation is repeated two or three times until finally the solution is allowed to stay on for two minutes before pouring off. The film is then washed in absolute alcohol for not more than 30 seconds. After draining off the alcohol the slide is quickly washed for a few seconds in distilled water, dried by waving to and fro in the air, and the counterstain applied, consisting of Bismark brown 3 parts in alcohol 30 parts, to which mixture hot distilled water 70 parts is added and the mixture let cool. The counterstain should not be applied to the film for more than thirty seconds for fear the purple bacteria may take on the brownish color. The pus corpuscles, epithelia and gonococci appear brown, but the other organisms remain purple. Wash the film a few seconds in water, dry, mount in balsam, and study with $^1/_{12}$ inch oil immersion lens.

☞ W. Henry Wilson uses a hot aqueous solution of Bismark brown without alcohol.

In a few cases of men with chronic urethral infections a diplococcus occurs resembling the gonococcus and only distinguishable by culture methods.

☞ In the case of women culture methods may reveal diplococci resembling the gonococcus in all other respects. The gonococcus in the case of women must be found in abundance in discharges from the cervix uteri, Bartholinian glands, Skene's glands or from the urethra in order to be of clinical value. Vaginal discharges in the case of women are almost worthless for clinical identification of the gonococcus. The same may be said of the urine of women.

Other Organisms.—Actinomyces bovis has been found in urine. It is also known as **Streptothrix actinomyces,** and is an organism more closely related to moulds than to bacteria, but its botanical

position is not yet accurately established. As encountered in the tissues and secretions of those infected it appears as tangled masses of threads the ends of which make a kind of radiated figure, or roset, and which are ordinarily visible in the form of small grains of a yellowish, red, or green color. At the extremities of the threads, particularly those of the periphery, pyriform or globular swellings are usually seen, while at other times nodular thickenings appear. The whole radiate mass is composed of this tangle of branching filaments constituting a mycelium, the filaments being sometimes composed of a succession of short rod-like structures. Primary actinomycosis of the kidney resembling tuberculosis as to mode of infection, clinical features, and anatomical distribution is known to occur.

Under the microscope the organisms appear as a dense group of radiating filaments with more or less bulbous ends, hence the term "ray fungus."

C. Heitzmann has described a case of **leptothrix** in the urine, slender thread-like micro-organisms being voided. Since, however, these occur in the mouths of healthy persons they might be found also in cases where patients spit into the urine. In case they are found in the urine, therefore, inquiry should be made as to their presence in the throat and mouth. They take a dark color with Lugol's solution.

The lactic acid bacillus of Boas has been found in a cancer nodule in the kidney in a case of stomach cancer.

Parasites and Worms.—Among the parasites found in the urine are the filaria sanguinis hominis, and distoma hematobium. Echinococcus, possibly eustrongylus gigas and fecal or vaginal parasites (ascaris, oxyuris, trichomonas). Nematode worms other than filaria, as *e. g.*, the "vinegar eel," have been reported.

Filaria sanguinis hominis (Fig. 57) is the parasite of *chyluria* found in the blood and urine of those passing milky urine. There are five or six species included under this designation. Some are found in the blood at night, others in the day, and still others all the time.

In this country filaria sanguinis hominis nocturna occurs **in**

Virginia, South Carolina, and Alabama. The parasites appear in the cutaneous circulation about five or six in the evening and increase in number until midnight.

They may be found both in the urine and in the tears. The embryos are about 0.3 mm. in length and are transparent, with pointed tails and enveloped in a sheath which is longer than the animal and projects beyond it at one or both ends. The parasites gain access to the stomach from drinking water, bore through the tissues, become sexually mature in the lymph vessels and their embryos get into the circulation through the thoracic

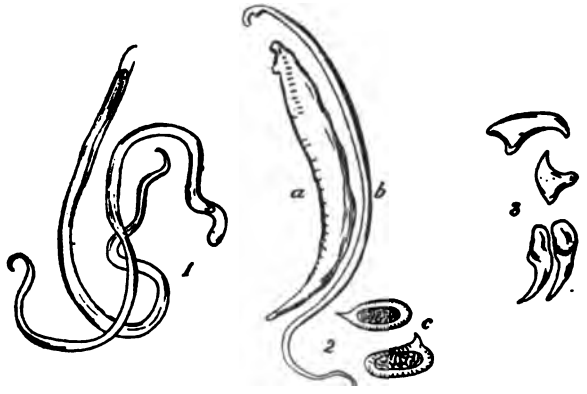


FIG. 57.—Animal Parasites: 1. Filaria sanguinis; 2. Distoma hematobium, *a*. male, *b*. female, *c*. eggs; 3. Echinococcus hooklets. (Croftan's original drawing.)

duct. In any case where the embryos are found in the urine or where bloody chylous urine (hematochyluria) occurs, the blood should be examined for the parasites. Patients presenting apparently inguinal hernia may in reality have a lymph scrotum due to filariasis, and in such cases the urine should not be overlooked. The occurrence of blood and chyle in urine (hematochyluria) is due to rupture of the varicosed lymph vessels of the bladder, these forming much of the collateral circulation which compensates for an occluded thoracic duct. The attacks may occur for as long even as eighteen years, each being weeks or months in duration and

separated by intervals of months or years. They come on either spontaneously or after exertion, excitement, etc. The onset is with pain and fever. First there is blood (hematuria), then blood and chyle, finally chyle alone. The embryos are then found in the urine, chiefly in the early morning.

Non-Parasitic Chyluria.—Chyluria is mostly called forth by the presence of parasites, especially of Filaria sanguinis hominis, in the blood or lymph. There are, however, cases in which there exists no parasitism. In such instances, it appears that there is an over-supply of chyle components in the blood which the latter cannot do away with. There are no gross renal lesions in these cases. The prognosis is not unfavorable. A case is cited by Huatek (*Deutsche med. Wochenschrift*, April 28, 1910) where a woman, thirty-three years old, suddenly voided milky urine. The urine exhibited 7.73 per mille albumin. Chyluria existed only at night; during the day the urine was not altered at all. When in the recumbent posture the urine contained also during the day 123 times the amount of fat which was excreted by the urine voided when walking. The left kidney eliminated four times as much fat as the right one. This is a symptom rather pointing to the cause as being situated in the neighborhood of the kidneys than to a disturbed renal activity per se. (Abstract in *Archives of Diagnosis.*)

Distoma hematobium, also known as schisostomum hematobium (Bilharz), is a *worm* common in Africa but rare in this country. The adule male (Figs. 57 and 59) is from 12 to 14 mm. long, flat and so folded as to form a gynecophoric canal which receives the female, 20 mm. long and filiform. "Egyptian hematuria" (bilharziosis) is the disease caused by this worm, which lives in the portal system, mucosa of the urinary tract and rectum, and in the pelvis of the kidney. The hemorrhages vary in amount from a few drops at the end of urination to profuse flow of blood. Pyelitis and atrophy of the kidney may be due to it and the eggs may form the nucleus of stone. The eggs may be found in the urine (Fig. 58).

Echinococcus (Hydatids).—Parasitic cysts of the kidney cause presence in the urine of the hooklets (Fig. 57) of Tænia echino-

coccus, daughter cysts, or fragments of the cyst membrane. Cysts of the same nature rupturing into the urinary tract may do the same. There are no symptoms of hydatid disease of the kidney unless there be present (a) catarrhal pyelitis or unless (b) the cyst rupture into the urinary tract. In the latter case the urine may be watery, soapy or bloody.

Tænia echinococcus is a small worm, 2.5 to 3.5 mm. in length. The head has a long prominent rostellum with a double circle of hooks, from 30 to 40 in number, and below the hooks are four suckers.

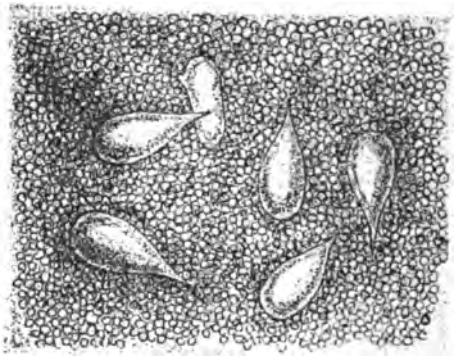


FIG. 58.—Eggs of distoma hæmatobium in sediment (after v. Jaksch).

The body is composed of only three or four segments, the last being equal in length to the others combined. The usual host is the dog. In man only the cyst form is known:—three varieties of cyst formation occur, the most common of which is known as echinococcus altriciparіens (E. hominis, E. hydatidosis). The embryo being set free in the stomach penetrates the mucous membrane and is carried to various organs in the blood or lymph current, where, finally lodged, the cyst develops about it. The cysts are formed in the liver, lungs, kidneys, spleen and in general in tissues having good blood supply.

The eggs are elongated, 0.12 by 0.04 mm., with a spine either at one pole or placed laterally. According to Emerson the condition has been found only six times in this country. One case

occurred in Chicago in a donkey-boy from Egypt during the World's Fair.

The eggs are found either free or entangled in clots of blood and mucus in the urine, and it has been estimated that from 3,000



FIG. 59.—Schistosomum hæmatobium, adult worms. (Copied from Braun). (Emerson.)

to 4,000 eggs may be voided in the 24 hours' urine. They may produce a sharp pain in passing through the urethra. The condition is more common in women and occurs between the ages of 10 and 50. The cyst is small at first and its wall consists of two

layers. Small buds or brood capsules appear in a few weeks, projecting from the inner layer; these become hollow in the center and from them the tape-worm heads (scolices) grow some 15 or 20 per capsule. The cyst increases in size by the growth of capsules and the cyst cavity contains *hydatid fluid,* colorless, odorless, neutral in reaction, of specific gravity, 1006-1015. The solids in it vary from 1.2 to 1.4 per cent. and consist of sugar and inosite, traces of urea and creatine, sodium chloride, and other salts. Hence when the cyst ruptures into the urinary tract a watery or soapy urine may be voided as described above.

In such urine the hooklets (Fig. 57) and other elements of echinococcus should be looked for. The condition is not common, only 100 cases or more being on record, 33 of which were in New York.

Eustrongylus visceralis (*E. gigas*), the giant strongyle, is a long worm found in the urinary organs of mammals and especially in the dog. Only 9 or 10 cases of presence of it in man are on record. The body of the worm is reddish and cylindrical, the male being from 15-35 cm., the female 25 cm. to 1 meter. The male has a single spicule at the end of a copulatory pouch which is easily recognizable. The kidney may be completely destroyed by this parasite. Blood clots from the urethra have been mistaken for this worm, which, in very rare instances, has actually been found in the urine.

Ascarides worms may be found in urine due to accidental washing away of one of the worms from the anus or to some fistulous opening between the intestines and the urinary tract.

The **oxyuris vermicularis** (pin or seat-worm) may escape from the anus and penetrate to the urethra, later being washed out in the urine. When such a worm is found in the urine identification is easy if the presence of seat-worms in the anus be established. These worms are small, the male 4 mm. in length and the female 10 mm.

Nematoids, not filaria, are occasionally found in urine, as, for example, **the vinegar eel, Anguillula aceti.** It is claimed that this worm has been found in the bladder. In other cases it is more

likely to be due to the previous contents of the receptacle in which the urine has been collected.

Anguillula aceti is a small hair-like worm found not only in vinegar but also in sour paste and similar substances. It is also known as Leptodera oxophila and Anguillula antiglutinis. It resembles the strongyloides of the intestines, but is slightly longer, the male being 1.2 mm. long and 0.033 wide; the female, 1.9 mm. long and 0.06 wide, and the embryo, 0.25-0.3 mm. long and 0.015 wide.

Vaginal Parasite.—The trichomonas vaginalis is found abundantly in the acid secretion of catarrhal vaginitis and may be found in the urine.

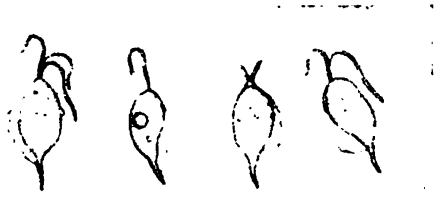


FIG. 60.—Trichomonas vaginalis (Emerson).

This parasite (Fig. 60) is from 15 to 25 microns long, from 7 to 12 broad, with its posterior end drawn to a thread, its cuticle thin, protoplasm free from granules. It has three flagella, as a rule, which sometimes seem united at base, the fourth, which is sometimes described, probably being the edge of the undulating membrane. These are of equal length. The undulating membrane extends spirally backward from the anterior pole. (Emerson.)

Objects accidentally occurring in urine (Fig. 61) found only after it is collected in a vessel and not occurring in the bladder or elsewhere in the urinary tract are likely to confuse the inexperienced. These may be derived from the air, from unclean vessels used in collection, from the external genitals, feces, saliva, or sputum, and from the clothing of the patient.

☞ **Fibers of textile fabrics** include cotton, linen, wool and silk. The most prominent and strongly refracting are *cotton fibers,* which are coarse and sometimes twisted. Their edges are never compact in the middle, which is wrinkled and irregularly striated.

Linen fibers are straight, not strongly refractive, have finer fibrils and have been hackeled, showing breaks and breaches. Irregular transverse breaks appear at different parts of them.

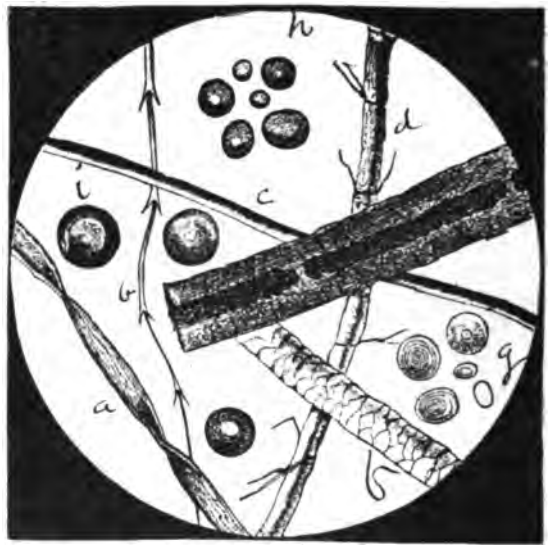


FIG. 61.—*a,* Cotton; *b,* feather; *c,* silk; *d,* hemp; *e,* hair follicle; *f,* wool; *g,* starch; *h,* fat; *i,* air bubbles. (After Kratschmer-Senft.) (Casper.)

The outer fibrils are often broken off and branch irregularly from the main fiber.

Woolen fibers have fine serrations along the edges, while the cuticle is serrated like the scales of some amphibians.

Silk fibers are smooth, homogeneous and shining, with jagged ends.

Feathers consist of fine quills with barbules composed of links of different sizes, gradually tapering towards the ends.

Hairs are very large, often of dark yellow or brownish color,

and extremely regular in outline, straight and at one end may show a characteristic widening due to the bulb. They have a central medullary canal. It must not be forgotten that persistent presence of numerous hairs in the urine may indicate a *dermoid cyst* of the urinary tract, as for example in the bladder. In such a case the hairs are found on catheterizing as well as in the collected urine, and are likely to be reddish in color. The formations thus far described being longer than they are broad are likely to be mistaken for tube-casts, and the beginner must be thoroughly conversant with the appearance of them.

Scales from insect wings appear as plates with small stems, and are transparent and delicate.

☞ Very common in the urinary sediment are **algae** from the water supply, *i. e.*, plant contaminations found in vessels used for collecting the urine, which have been rinsed out with water. Some of these are longer than broad and may be mistaken for tube-casts, but being strongly refractive, segmentated, or striated, should cause no confusion. Many of these algæ have a yellowish color. They have not received the attention in the smaller books to which they are entitled as a source of confusion. The first slide shown the author by the late Charles Heitzmann was one containing algæ from Croton water, which were different in appearance from those occurring in Lake Michigan water. Students are likely to assign more importance to these plants than they deserve as they are of no clinical significance. They occur in various shapes, as shown in the figure, which, however, fails to indicate the prominence of them in the microscopic field, as they refract strongly. Emerson shows them admirably in an illustration (Fig. 62) in his Clinical Diagnosis.

Cork particles are irregular, yellowish, or reddish brown, often grouped in masses and strongly refractive.

Rust particles look like hematoidin crystals, but are larger and more irregular.

Globules are of various kinds.

Air bubbles are the most common and are large, circular, sharply defined with double contour and a bluish-black refraction.

Oil globules are of circular shape and of different sizes, yellowish in color and strongly refractive. Large ones indicate use of oiled catheter, passage of oiled instruments, etc. The ap-

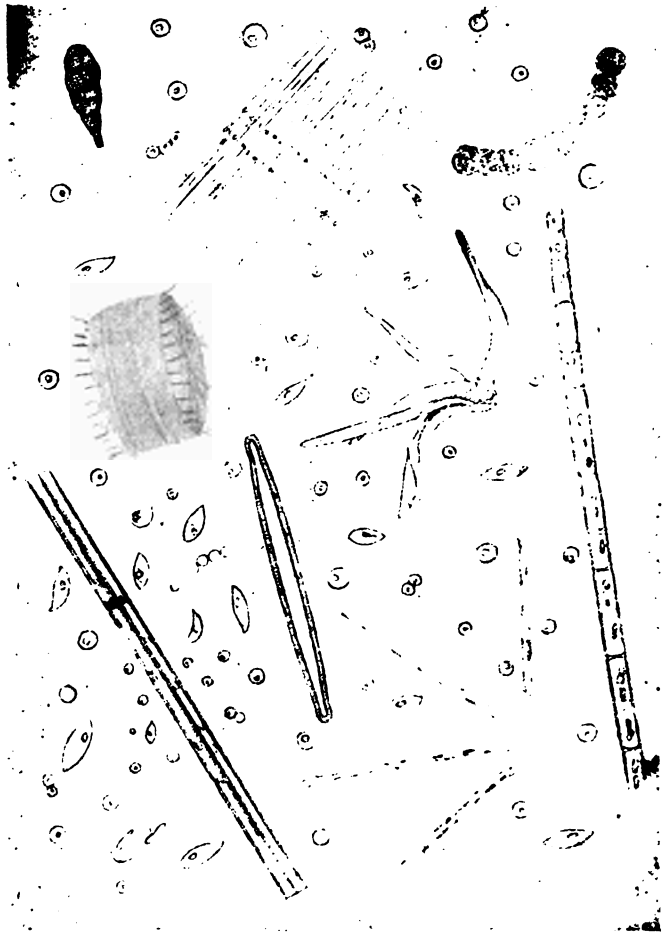


FIG. 62.—Protophytes and other low forms of life often found in tap water. × 400. (Emerson.)

pearance of oil in the urine may be studied by mixing vaseline or olive oil with the sediment.

Starch globules are common owing to the use of dusting powders. They are roundish or oval, refract strongly and have a central hilum with concentric striations. Rice-starch, wheat-starch or corn-starch may be present.

Lycopodium powder is also used for dusting and is found in urine in the form of roundish shells filled with many spherical particles.

Cellulose appears in the form of a framework of cells with straight lines bounding the several cells, the latter being angular or rectangular and containing large oblong nuclei.

Feces in the urine are best recognized by the fecal odor. It will also be possible to find with the microscope particles of fecal matter consisting of vegetable matters, cellulose, starch, fat globules, spores, muscle-fibres, connective tissue, triple phosphate crystals, bacteria, fungi, yeast, etc. Bacteria in enormous quantities are present in feces and hence may be abundant in fecal urine.

The significance of feces in urine is that of involuntary stool or recto-vesical fistula, or rectal cancer, when, in addition, masses of tumor cells and pigmented epithelium from the intestines will be found.

☞ *Flaws in the glass* are puzzling to beginners, as are also scratches on the slide. They are readily distinguished from the sediment by their fixed position when the sediment is floating about and moving.

Dust, etc., on the eye-piece is readily recognized by turning the eye-piece round while the observer is studying the sediment. It will then be seen that the misplaced matter moves with the eye-piece.

CHAPTER XXX.

THE DETERMINATION OF THE RENAL FUNCTION. TOXICITY OF URINE.

Ureteral catheterization.
Measuring the ratio of excretion of the kidneys.
Methylene-blue: technique of the test.
Indigo-carmine: dose and technique of the test.
Phenolsulphonephthalein test.
Measuring the chemical activity of the kidneys.
Phloridzin test: dose and technique; errors.
Experimental polyuria test.
Toxicity of urine; the urotoxic coefficient.

In order to safeguard the patient from the effect of surgical operations which have to do with the removal of one kidney, tests have been proposed, the object of which is to determine the functional value of each kidney beforehand. The urine from each kidney may be obtained separately by the use of the ureteral catheter or in the bladder by means of the segregator.

Koranyi believes that through the glomeruli are **filtered** the urinary water and salts, but that other constituents (urea, etc.) are **secreted** by the tubular epithelium, as according to previous theories, but he holds that a perfect balance is maintained between the number of molecules secreted and those filtered, and that for every molecule of urea, etc., secreted, a molecule of sodium chloride, etc., is reabsorbed by osmosis through this same epithelium. Hence changes in the epithelium due to disease will be shown by changes in the ratio of urea to the various salts.

To determine the functional activity of the kidneys a number of methods have been proposed, none of which is infallible. Cryoscopy and determination of electrical conductivity have already been described. Neither is now popular. These methods seek to determine the molecular concentration of the urine. To measure the ratio of excretion of urine various dyes are used, as methylene-blue, indigo-carmine, and phenol-sulpho-nephthalein.

Methylene-blue (chemically pure medicinal) is injected into the muscles in doses from 0.02 to 0.05 gramme. Normal kidneys begin to excrete the dye within 15-20 minutes and finish in 48 hours. It should not be delayed longer than 30 minutes and should be at its maximum in the third or fourth hour.

Methylene-blue is no longer popular; the fact that it may be excreted as a colorless chromogen or that other changes in it may take place in the body is against its use. Other objections to it also exist.

☞ **Indigo-carmine** is, on the whole, the best dye for the purpose, as it is rapid in appearing, of constant color, of intense color, shorter in being eliminated, readily used, and better suited for deducing conclusions. The usual dose is 4 c.c. of a 4 per cent. suspension of the dye in normal salt solution, 4 inches above the knee-cap, injected into the quadriceps muscle. The ureteral catheters, if used, should be in their place before the injection, but it is not necessary, in most cases, to use these at all. Healthy kidneys begin to excrete indigo-carmine in about five minutes. The color is most intense in about 30 to 45 minutes and lasts about 12 hours. If the color does not appear for 10 or 12 minutes after injecting 0.16 gramme of the dye and there is only a green, and if the color never becomes blue, renal insufficiency is indicated. The intensity of the color is held to be of value and can be determined by use of the chromometer of Duboscq by diluting the urine with normal urine.

Delayed excretion and lessened excretion mean functional renal disturbance.

☞ **Phenolsulphonephthalein** is a bright red crystalline powder somewhat soluble in cold water. The subcutaneous dose is 6 milligrammes dissolved in one cubic centimeter of water to which a drop or two of half normal sodium hydroxide solution has been added until the color changes to a Bordeaux red, when the substance is not irritating when injected subcutaneously. The patient should drink 300 c.c. of water 20 or 30 minutes before the injection is made. Under aseptic precautions a catheter should be passed and the bladder completely emptied or, if the case is a

prostatic one, previous gradual withdrawal with a course of urotropin should have been practised. The time is then carefully noted and a subcutaneous injection of six milligrammes in 1 c.c. of water made into the tissues of the upper arm.

The end of the catheter is placed in a test-tube containing a drop of 25 per cent. sodium hydroxide solution and the time of the appearance of a faint pinkish tinge noted. The catheter is then withdrawn or closed and at the end of the first hour the urine is voided or withdrawn; this is repeated at the end of the second hour.

If ureteral catheters or segregators are used, continuous drainage is allowed into separate receptacles for the first and second hour periods. The volume of each hour specimen is carefully measured, the specific gravity taken, and the urea estimated. Enough 25 per cent. sodium hydroxide solution is added to make the urine decidedly alkaline, in order to obtain the maximum color. This solution is then placed in a liter flask and made up to one liter with distilled water, mixed well, and the colorimetric process with the Duboscq colorimeter used for determination of the percentage amount of drug passed. Normally, the first appearance of the drug is in from six to eleven minutes and from 60 to 85 per cent. of the drug should be eliminated in two hours.

The permeability of the kidneys to this drug is affected in both interstitial and parenchymatous nephritis especially in the latter. Pyelitis may be differentiated from pyelonephritis by use of the method.

☞ The **chemical activity of the kidneys** is measured by the phloridzin test, which produces in normal persons glycosuria. Certain structural changes, *i. e.*, interstitial, in the kidneys, prevent the glycosuria.

The test is made as follows: 1 gramme of phloridzin is dissolved in 30 c.c. of 95 per cent. alcoholic and 70 c.c. of warm water added. Of this freshly made warm 1 per cent. solution 15 minims are injected hypodermically into the arm, not too deep. If the kidneys are normal, sugar appears in the urine in from 10 to 15 minutes after the injection, reaches a maximum at the

end of 90 minutes after the injection, and disappears in about 12 hours.

If the appearance of sugar is delayed to 20 minutes or more, there is renal insufficiency, and if none appears within 45 to 50 minutes the insufficiency is serious.

In using phloridzin it is necessary to obtain the urine by means of the separator or ureteral catheters. The latter are inserted before the injection and the urine allowed to drop through catheters into graduated test-tubes.

The amount collected for the first 10 minutes is poured off for other tests, while that obtained at the end of 15 minutes is tested for sugar, and so on. The time of the appearance of the sugar is held to be of the most importance.

There are said to be grave errors in the phloridzin test as applied to normal kidneys and in general the indigo-carmine test is preferable. Phloridzin is said to be of the most value in chronic interstitial nephritis, but inasmuch as failure to excrete sugar has been noted in comparatively healthy kidneys, use of this test is not so common as it was at one time.

Experimental polyuria is a term applied to a test for the functional capacity of the kidneys, proposed by Albarran. From 400 to 600 c.c. of Evian (mineral) water are given the patient and in healthy kidneys a marked lowering of the specific gravity is noted; in diseased kidneys the specific gravity is lowered less and more slowly; in chronic interstitial nephritis no lowering of the specific gravity takes place and practically no polyuria.

It is said that this method also is fallacious for a number of reasons.

Of the various methods proposed the indigo-carmine and phloridzin are probably now the most used, and the former is the more practical.

Alimentary Salt Excretion Test.—Place the patient upon three liters of milk per 24 hours, and after the third day add to the diet sodium chloride, 10 grammes dissolved in 125 c.c. of water, given in three portions on each of four consecutive days with daily determinations of the amount of chlorides.

This test is supposed to show the glomerular efficiency of the kidneys since normally the excretion begun at once and ends abruptly when ingestion of salt ceases. If the excretion of the salt is delayed, reaches a slow maximum, and continues two to four days after ingestion ceases, or if there is no increase in excretion, the condition of the kidneys is either serious or hopeless. But the test is not popular, being subject to many exceptions.

H. Strauss uses this test in the diagnosis of diabetes insipidus in which he finds distended bladder.

Toxicity of Urine.—Various observers have noticed that normal urine is poisonous to animals. Bouchard claims a *urotoxic coefficient* which represents the weight of kilogrammes of rabbit killed by the quantity of urine excreted by one kilogramme of person per 24 hours. A man weighing 60 kilogrammes and passing 1200 c.c. of urine in 24 hours secretes enough poison to kill 24 kilos of rabbit when 50 c.c. are necessary to kill one kilogramme.

But according to Marfan the coefficient in health ranges from 20 to 35. The poisons to which the toxicity of urine are supposed to be due are ptomaines and leukomaines, the former of bacterial action (putrefaction), the latter of chemical (retrograde metamorphosis).

The purine bodies are leukomaines, as also are the creatinine group of substances.

Nothing practical has so far resulted from observations on the toxicity of urine, but as ptomaines and leukomaines are the chemical bases of infectious diseases, it is hoped that from the urine may some day be isolated substances which specifically cause these diseases.

CHAPTER XXXI.

URINARY CONCRETIONS.

Sand, gravel and stones: situation and size, number, composition, constitution, nuclei, crust, surface, shape, color and hardness.

Pathology of calculi: causes favoring the growth; effects of calculi; cystine stones.

Different kinds of calculi: uric acid and urates: properties and tests; oxalate calculi: properties and tests; complete analysis; phosphatic concretions: properties, tests, complete analysis; cystine calculi: properties, tests, complete analysis.

The rare calculi: xanthine concretions, calcium carbonate stones, protein concretions, mixed concretions, indigo calculi, fatty concretions.

Physical and chemical analysis of calculi: the physical characteristics; charring without flame; absence of residue; presence of residue; solubility of residues; meaning of effervescence with acids; separation of constituents according to charring.

Complete systematic analysis: solution in dilute hydrochloric acid; treatment of residue; treatment of hydrochloric acid solution.

URINARY CONCRETIONS.

Calculi which come from the pelvis of the kidney and ureter are called renal stones. They may also be found in the substance of the kidney. According to *size* they are termed sand, gravel or stones. Large ones may fill the entire pelvis of the kidney, or even practically replace the kidney. Renal stones, weighing as much as half a pound have been seen by the writer. Stones with branches which may be hollow, allowing the passage of urine, may form casts of the pelvis and calices of the kidney. They may cause pyelitis, pyonephrosis, and ulceration of the pelvis or ureter. They may perforate into the bladder, intestines, peritoneum or cellular tissues; calculi may be formed in sinuses connecting the urinary passages with the intestines, uterus or vagina, and they may occur also in the seminal vesicles. Stones may form also in the bladder, urethra and prostate.

As a rule, the renal stones are smaller and bladder stones larger.

A stone weighing nearly four pounds (1,596 grammes) is on exhibition in Paris. In general, the size is limited only by the dimensions of the cavity in which it is formed, varying from that of a pin head to that of an orange.

The *number* varies from one to hundreds.

Composition.—Calculi may be composed of almost any of the constituents of the urinary sediment. They are formed of material supplied by the kidneys, and, as a rule, have an organic nucleus. Those which separate from the urine as a result of ammoniacal decomposition are known as *secondary concretions,* and are composed of triple phosphate, calcium phosphate, calcium carbonate and ammonium urate, while those forming in unaltered urine are *primary concretions,* and consist, as a rule, of urates and oxalate. Other primary constituents are uric acid, calcium phosphate and carbonate, cystine, xanthine, indigo, fatty (urostealith) silica, protein (albumin, pus, blood) and bilirubin or hematoidin.

The commonest stones are the urate and the oxalate.

Primary stones may cause decomposition of the urine and become crusted over with triple and other phosphates. Foreign bodies in the bladder, as hair-pins, etc., may become similarly crusted.

Ammonium urate may form primary stones in new-born children. Stones may be *simple* in constitution, consisting of only one ingredient, as uric acid or calcium oxalate, or *compound,* consisting of two or more, occurring in separate and alternate layers, most commonly uric acid and oxalate. Several constituents may be mixed together in any part of the stone. Stones, as a rule, consist of a nucleus and a body, but in addition there may be a crust. The nucleus is usually a protein substance, especially coagulated fibrin, morbid tissue or a mixture of mucopus with crystals, but sometimes the nucleus is of the same composition as the rest. The nucleus may, in the bladder, be a foreign body. A stone may have several nuclei, as when several stones have united to form one. The nucleus varies in size, and is usually in the center, but if the growth of the stone is in one direction only it may be placed eccentrically.

The body of the stone surrounds the nucleus and forms the greater part of the stone. It usually consists of concentric layers of two or more urinary constituents, as one of uric acid and urates, another of oxalate, etc.

The crust is absent unless ammoniacal decomposition has taken place, hence always consists of secondary constituents, *i. e.*, mainly phosphates. In the case of smooth stone no crust may form, even in alkaline urine.

The surface of a stone may be either rough or smooth. Oxalate stones are usually rough and nodular, while other stones are more or less smooth. Multiple stones may rub against one another and form *facets, i. e.*, smooth, polished surfaces.

The **shape** of the stones depends on the location and size. Small concretions in the pelvis of the kidney are round or oval, large ones branched or like an elephant in shape. In general those from the kidney tend to be irregular in shape.

Calculi in the bladder are, if single, round, oval or flat; if multiple, changed by pressure. Encysted stones in the bladder grow in only one direction, and have a very irregular shape. Stones forming in the urethra are generally oblong or cylindrical, and, if multiple, have adjacent ends highly polished.

The **color** varies with the constituents. Uric acid and urate stones are always colored, varying from light yellow to dark brown; oxalate are dark brown, phosphatic white or grayish; cystine yellowish; indigo bluish; calcium carbonate whitish; xanthine reddish-yellow; gall stones have been found in the bladder in cases of fistulous openings.

The dark-brown color of oxalate stones is due to presence of decomposed blood and fibrin.

The **hardness** varies with the composition, the oxalate being the hardest and the phosphate the softest.

Pathology.—The causes favoring the growth of calculi are numerous, but the commonest are: (1) Undue acidity of the urine or alkalinity of it, the former favoring deposits of uric acid, urates and oxalate, while the latter causes separation of phosphates, carbonates and ammonium urate; but urine alkaline from fixed alkali does not favor the formation of stone; (2)

diminution in the volume of urine, especially when coupled with increase in the slightly soluble urinary constituents. In such cases uric acid, acid urates, oxalate, cystine and xanthine may be deposited.

Stones may form anywhere in the urinary tract. In the bladder foreign bodies known to have become nuclei for stones are peas, beans, catheter or bougie fragments, soap or candle fragments, hair pins, pins, needles and bullets.

☞ The drinking of lime water, diet, hereditary predisposition, sex, and time of life are thought to favor formation. Stones are more common in males, and in those less than twenty years of age. They are more common in children.

The effects of renal stones have already been mentioned. Bladder stones cause pain, frequent and painful urination, hematuria, cystitis, hypertrophy of the bladder, pyelitis and pyelonephritis (surgical kidney).

Patients subject to cystine stones lead a life of misery from recurrence of calculi, perhaps necessitating repeated operation. Other calculi do not necessarily recur.

☞ **Uric Acid and Urate Concretions.**—The large majority of concretions found in the urinary tract are composed of uric acid and urates, especially in the calculi of children, which are chiefly ammonium urate. They are always colored, having a tint varying from grayish-yellow to yellow-brown or light red-brown. They are usually smooth, round or oval, but sometimes may be rough and nodular.

They are always very hard but fracture easily, and on cross-section show a concentric arrangement and crystalline structure, the layers of which may be separated, being of different colors. When from the kidney they vary in size from a pin head to that of a kernel of wheat, or of a pea. When they are retained in the bladder they grow more or less rapidly, varying in weight from a few grains to several ounces, and may become rough or irregular. Concretions consisting chiefly of urates, however, do not attain the large size of the mixed uric acid and urate calculi, seldom being found larger than a marble of average size. They are, moreover, lighter in color and not so hard as the mixed

concretions. Ammonium urate stones are waxy in consistence, and, when dry, clayey and easily powdered. Calculi composed of uric acid or ammonium urate char and completely disappear when powdered and heated on the platinum foil. If sodium urate is also present, the residue is sodium carbonate soluble in water and of alkaline reaction.

The original powder is insoluble in warm dilute hydrochloric acid, and gives off an odor of bitter almonds when heated to redness on the platinum foil. The original powder also yields the murexide reaction. Uric acid concretion liberate but little ammonia with sodium hydroxide, while ammonium urate stones liberate much. When these stones are forming the urine is likely to be concentrated and acid with deposits of uric acid or urates or both.

☞ **Oxalate calculi** are very hard, heavy, of a mulberry-like surface (mulberry calculi), of medium or large size, dark-brown or dark-gray, irregular, rough and trabeculated. They can be crushed only with difficulty. When cracked or sawed in two they show crystalline structure. Occasionally they occur as small, white, smooth stones ("hempseed" calculi). The larger stones may be white or yellowish in color, may show angular protuberances, and are not infrequently covered with blood-pigments or urinary coloring matters. They cause severe symptoms and hemorrhage due to them is common. Oxalate concretions are composed chiefly of calcium oxalate mixed with more or less organic matter, or there may be alternating layers of oxalate and uric acid. The nucleus often consists of uric acid and urates, coagulated blood and mucus or entirely of oxalate. In some cases the concentric layers are composed of uric acid, xanthine or calcium carbonate. Many oxalate stones have a phosphatic crust. The urine is usually acid and concentrated, when these stones are forming and the sediment is likely to contain oxalate crystals. When oxalate stone is forming in the bladder, a typical chronic cystitis may exist.

Powdered and heated on platinum foil a white residue remains after a slight charring of the organic matter. The white residue is calcium oxide or carbonate according to the amount

of heat used. If the former it is slightly soluble in water, to which it gives an alkaline reaction; if the latter it is soluble with effervescence in acetic acid.

The original powder is soluble in hydrochloric acid without effervescence but not in acetic acid. After moderate heating, however, the powder is converted into calcium carbonate, which is soluble in acetic acid with effervescence.

For complete analysis the concretion is powdered, repeatedly treated with warm hydrochloric acid, and filtered. The solution is carefully neutralized with sodium carbonate until a slight precipitate forms, this is redissolved in the smallest possible quantity of hydrochloric acid and the liquid treated with an excess of a 30 per cent. solution of sodium acetate; the precipitate which occurs consists of calcium oxalate or cystine. It is filtered off and washed off the filter. If it consist of oxalate alone without cystine, it is insoluble in ammonia and insoluble in acetic acid, but soluble in hydrochloric acid. Supersaturate the solution in the last named acid with sodium acetate and a flocculent precipitate of calcium oxalate again forms. Collect this on a filter, wash off and heat moderately on platinum foil, when it will develop carbon dioxide on treatment with acetic acid. The solution in acetic acid will again yield a precipitate with ammonium oxalate.

☞ **Phosphatic concretions** are rare and small in the kidneys but more common in the bladder, where they are larger and may be very large in size. They are usually light in weight, gray or whitish, pale yellow or purplish, soft and easily crushed. Their surface may show glistening points, due to large crystals of triple phosphate. The surface may be smooth and chalky or rough. The grayer the color the harder the stone, since the former are mostly calcium phosphate and the latter triple. Phosphatic calculi consist for the most part of a mixture ot earthy phosphates and triple phosphate. Concretions consisting of earthy phosphates alone are rare, are white and of a handsome crystalline structure. Concretions of triple phosphate alone are rare, small and have a granular surface on which may be reddish crystals.

Phosphatic calculi (mixed phosphate) may also contain am-

monium urate, calcium oxalate and carbonate. They are likely to exist as a crust upon other stones and to form in the bladder round some foreign body. Urine in which they are forming is neutral or alkaline from volatile alkali. The powdered calculus does not char nor burn when heated, but leaves an abundant residue soluble like the original powder in acetic acid.

The complete analysis is pursued as in the case of oxalate above. The filtrate, after addition of sodium acetate, is divided into two portions and treated with ammonia and ferric chloride solution (20 per cent.). Both cause cloudiness or precipitates if phosphates are present. Another portion of the same filtrate if treated with ammonium oxalate solution yields a fine whitish precipitate soluble in dilute hydrochloric acid if calcium is present. Magnesium in the concretion is detected by precipitating all the calcium with ammonium oxalate, filtering, evaporating the filtrate to a small volume, and treating with one-third its volume of 10 per cent. ammonium hydroxide. A crystalline precipitate of triple phosphate shows magnesium in the original. Ammonium is shown by adding to the original hydrochloric acid extract (see Oxalate above) sufficient sodium hydroxide to make it strongly alkaline and holding moistened red litmus over the mouth of the tube. Ammonia gas, if present, will turn the red paper blue.

The mixed phosphate calculi are known as "fusible calculi" since they melt in the blow-pipe flame.

Cystine stones are rare. They vary in size from renal concretions not larger than a small pea to bladder stones as large as a hen's egg. One such bladder stone is reported by Dr. E. S. Wood, weighing more than 100 grammes, in the form of a flattened oval. Cystine stones are generally of light weight, quite soft, of white or pale yellow color, oval or cylindrical in shape, and may have either a smooth or a rough finely granular surface. On cross-section they are crystalline and present a radiating appearance. They appear yellow like beeswax when first taken from the body, but on exposure to the light change to green. They burn readily and completely with a bluish flame when heated on platinum foil, and an odor of sulphur is given off. No residue remains after ignition.

The powdered concretion is soluble in ammonia and in mineral acids, but insoluble in acetic acid. If the powder is dissolved in ammonia and the mixture is allowed to stand upon a slide until the ammonia evaporates, hexagonal crystals of cystine will be found by the microscope.

The powder, boiled in a solution of lead oxide in sodium hydroxide, is blackened owing to liberation of sulphur and formation of lead sulphide. The lead solution may be made by adding sodium hydroxide solution, drop by drop, to lead acetate solution until the precipitate formed is dissolved.

The complete analysis proceeds as under Oxalate above, when the precipitate formed by sodium acetate is filtered off and treated with ammonia and the solution formed may be identified as follows: First, addition of acetic acid to it causes a crystalline precipitate of cystine; second, if this precipitate be filtered off and washed off the filter into a lead solution as above, boiling will cause development of a black color in the liquid.

Xanthine concretions are very rare and occur mostly in children. They vary in size from that of a pea to a hen's egg, vary in color from pale-white to pale-yellow to a cinnamon-brown, are rather hard, rub like wax, then become shiny and are amorphous in cross-section. They burn without residue, after charring, resembling uric acid. When the murexide test is applied to the powdered concretion a pink tint appears, which deepens to a violet on warming, while uric acid gives a violet which disappears on warming.

For complete analysis powder the calculus and treat repeatedly with dilute warm hydrochloric acid, supersaturate with ammonia water and add ammoniacal silver nitrate. A precipitate occurring indicates presence of xanthine.

Calcium carbonate stones are rare, chalky-white or gray, small, round, smooth and very hard. On section they show concentric layers. The powdered concretion is soluble in acetic acid with effervescence. Heated to redness on platinum it is reduced to oxide, which is but slightly soluble in water, to which it gives an alkaline reaction.

Heated on the platinum foil the fatty calculus burns with a

yellow flame and gives off an odor of shellac or benzoin. The calculus may be also recognized by the "grease spot" test on paper when warmed.

Protein Concretions.—Fibrin or blood concretions result from coagulations of the blood within the urinary tract and may be either nuclei of other calculous growths or not infrequently contain a deposit of uric acid, oxalate or phosphates. Heated they burn with a yellow flame and yield an odor of burnt horn. Hematoidin crystals may occur in concretions, most commonly in fibrin ones. One calculus composed of (coagulated?) albumin has been reported.

Prostatic concretions consist of amyloid bodies (corpora amylacea) about which are deposited mixed phosphates. They are rare and cause no disturbance until large.

Mixed concretions may occur, containing in addition to the various substances previously mentioned calcium carbonate and sulphate, leucine, tyrosine, bilirubin, hippuric acid, cholesterine and fats. Calcium sulphate may be recognized by barium chloride added to the hydrochloric acid solution. Ether will extract cholesterine and fats from the finely powdered calculus and the residue on evaporation when examined with the microscope will show the large rhomboid plates or fine silky needles of cholesterine. The crystals turn red and blue when touched with sulphuric acid.

Indigo concretions are rare, only two or three having been reported. They have a blue or bluish-gray surface or may be dark brown in color. Drawn across paper they may leave a rough blue mark. Those reported weighed from 10 to 40 grammes. Indigo may, however, serve as a nucleus for other stones.

Fatty concretions (urostealith) are also very rare. They contain free fatty acid, neutral fat, and much cholesterine, are soft and elastic when fresh, but when dry are hard and brittle. They are generally of a yellowish or brownish color and frequently inclosed within a phosphatic crust. In some cases they consist of the fat used in making bougies.

PHYSICAL AND CHEMICAL ANALYSIS OF CALCULUS.

☞ Note first the *physical characteristics* of the stone, *i. e.*, the color, size, shape, weight, hardness and appearance of cut section made with a jeweler's saw. If the stone is in different layers it is well to scrape off with a knife some powder from each layer and test it. The nucleus should also be separately examined whenever possible. Having reduced to powder in a small clean mortar the portion of the calculus to be examined, a portion of it should next be heated on platinum foil, and the kind of flame, odor and residue carefully observed. A blue flame with odor of burning sulphur suggests *cystine;* an odor of burnt feathers, *protein;* an odor of shellac and benzoin, *fat;* a bitter almond odor, *uric acid or xanthine;* charring without flame and leaving no residue, *ammonium urate, uric acid and xanthine;* more or less residue, *urates, phosphates, carbonate, oxalate, silica.* If the residue is insoluble in hydrochloric acid, *silica;* if the residue is soluble in water with alkaline reaction, *urates of the alkalies* (sodium and potassium); if the residue is soluble in 50 per cent. acetic acid, *earthy phosphates, oxalate, carbonate;* when the residue is soluble in acetic acid and the original powder is also soluble in acetic acid without effervescence, *earthy phosphates;* with effervescence, *calcium carbonate;* original powder insoluble in acetic acid, *calcium oxalate.* When some one constituent is thus suggested consult complete analysis described under the heading of this constituent.

It is also convenient to separate the constituents according to whether marked charring takes place or not. If the powder does not char appreciably, then salts of lime and magensia are the principal constituents, *i. e.*, calcium carbonate, oxalate, earthy phosphates and triple phosphate; add hydrochloric acid, and, if there is effervescence, *calcium carbonate* is present. If there is no effervescence on cooling, *oxalate* or *phosphates* are present, but if the original powder effervesces when gently heated with hydrochloric acid, it is *oxalate;* if not, then composed of *phosphates.* To distinguish earthy phosphate from triple moisten the original powder with a little potassium hydroxide solution and note odor of ammonia if it is *triple phosphate.*

If now the powder does char, note whether with or without flame: if with a flame *protein, fat* and *cystin,* distinguished by odor as above. If the powder chars without flame, uric acid, ammonium urate and xanthine are suggested, the two former being distinguished by the murexide test and *ammonium urate* differentiated from uric acid by the strong odor of ammonia when moistened with potassium hydroxide solution. If the powder leaves an abundant residue of ash that emits a white glow when strongly heated, it is composed of *phosphates.*

In difficult cases it may be necessary to make a complete systematic analysis, as follows:

Systematic Analysis.—1. Reduce the calculus to a fine powder and pour over it some water and finally dilute hydrochloric acid in a beaker. Warm gently half an hour, or longer, on the water-bath. Then allow to cool and filter.

2. Treatment of the residue. It seldom happens that the calculus is completely soluble in the weak acid. A residue usually remains which may contain uric acid, xanthine, calcium sulphate, and remains of organized matter. To prove the xanthine treat the residue with warm *dilute* ammonia and filter. The filtrate contains the xanthine if it is present. Acidify it with nitric acid and add a small amount of silver nitrate solution. This produces a flocculent precipitate which dissolves by warming, and crystallizes on cooling in bunches of fine needles.

In the residue free from the xanthine look for calcium sulphate by extracting with water and applying the usual tests. This solution may contain uric acid which is recognized by evaporation and crystallization after adding a little hydrochloric acid. In the final residue some uric acid may be also present. Dissolve in alkali, reprecipitate with hydrochloric acid, and examine any crystals which may form under the microscope.

3. Treatment of the hydrochloric acid solution which may contain calcium oxalate, cystine, the phosphates and possibly some xanthine. Look for the last in a small portion of the solution. Make this portion alkaline with ammonia, add a few drops of calcium chloride solution, filter if a precipitate forms and treat

the filtrate with ammoniacal silver nitrate solution. In presence of xanthine a flocculent precipitate forms.

Dilute the remaining and larger portion of the hydrochloric acid solution with twice its volume of water, add enough ammonia to give a strong alkaline reaction and then acetic acid to restore a weak acid solution. By this treatment phosphates are held in solution, while calcium oxalate, if present, precipitates. Therefore allow mixture to stand half an hour and then filter off any precipitate which appears. This precipitate may contain cystine as well as calcium oxalate. Cystine may be dissolved by pouring ammonia on the filter, and, on evaporating, the ammoniacal solution is obtained in form suitable for microscopic examination.

The residue free from cystine is dried and heated to redness on platinum foil. This treatment converts calcium oxalate into carbonate. Place the foil in a beaker and add some dilute acetic acid; an effervescence shows the carbonate. To the clear solution add next some ammonium oxalate which gives a white precipitate of calcium oxalate, if the latter metal is present.

Next look for phosphates and bases in the acetic acid solution obtained after filtering off cystine and calcium oxalate. More calcium may be present, in excess of that combined as oxalate, which may be recognized by adding a little solution of ammonium oxalate. If a precipitate forms treat the whole of the liquid with ammonium oxalate, after warming on the water-bath, allow to stand an hour and filter. Concentrate the filtrate to a small volume, transfer to a large test-tube and add enough ammonia to produce an alkaline reaction. If a precipitate now appear, it must consist of magnesium phosphate, showing both magnesium and phosphoric acid in the original. If no precipitate appear, magnesium is absent, but phosphoric acid may still be present. To find it divide the ammoniacal liquid into two portions. To one add a few drops of magnesia mixture (white ppt.) and to the other nitric acid in slight excess, and then a few drops of ammonium molybdate solution (yellow ppt.). Both tests must be successful. *Ammonium salts* are recognized by heating

the original powder moistened with strictly pure potassium hydroxide solution (odor of ammonia and red litmus paper turned blue). *Sodium* and *potassium* are recognized by treating the solution of the powdered calculus in hydrochloric acid with pure ammonia water and a slight excess of ammonium carbonate. Let the precipitate settle and filter. Evaporate the filtrate to dryness in a platinum dish and heat the residue strongly to drive off ammonium salts.

CHAPTER XXXII.

THE DETECTION OF DRUGS AND POISONS IN THE URINE.

Inorganic substances: tests for bromine, iodine (bromides and iodides), E. M. Bruce's test for iodine; mineral acids and caustic alkalies; metallic salts; potassium chlorate, lithium compounds, arsenic, lead and copper salts, silver salts; mercury compounds; iron salts, etc.

Organic substances: tests for alkaloids; morphine, nicotine, atropine, quinine, veratrum, strychnine; tests for coal-tar compounds: anilin, antifebrin, antipyrin, carbolic acid, eosin, kairin, naphthalin, methylene-blue, nitrobenzole, phenacetin, pyramidon, rosanilin, salicylates, thallin; tests for resins: copaiba, sandalwood oil, chrysophanic acid, santonin; tests for alcohol and similar substances: chloral hydrate, chloroform; tests for gallic and tannic acids: carbon monoxide, etc.

DETECTION OF INORGANIC SUBSTANCES.

Inorganic substances which may be found in the urine are halogens, acids and alkalies, and metallic salts, including those of arsenic and antimony.

Halogens.—Bromine and iodine in the form usually of bromides or iodides occur frequently in urine when administered internally in appreciable doses or when iodoform is used externally.

☞ **Iodine** is (1) readily detected when the author's method for indican is used, the chloroform becoming colored a bright pink.

☞ 2. E. M. Bruce, of Chicago, prefers the following test:—half a test-tube full of urine is strongly acidulated with hydrochloric acid to prevent precipitation of phosphates and a few drops of a weak solution of ferric chloride is added, after which 1 to 2 c.c. of pure carbon disulphide is added, with shaking. Chloroform may be used instead of carbon disulphide, but the latter is preferable and will show small amounts of iodine by the reddish color. Bruce finds this a useful test for estimating the amount of iodine removed from the system from day to day,

when sodium sulphide is given persons who need to have the iodine eliminated.

3. Iodine may be detected in the urine by adding to half a test-tube full 10 drops of yellow nitric acid and a few c.c. of chloroform with shaking. The chloroform is colored reddish or violet.

4. In the cold nitric-acid-by-contact test a red-brown color appears when iodine is present. But as more or less color always appears when this test is used tests 1 and 2 are preferable.

5. The starch test for iodine may be useful when much indican is present. Add a few drops of starch paste to the urine and float the latter upon pure strong nitric acid. A blue color forms at the zone of contact and is destroyed by heat. Or mix starch paste with the urine, add some dilute sulphuric acid and nitric acid when a blue or black color is formed, if iodine is present, and is destroyed by heat.

Bromine.—A ready clinical test is to acidulate with hydrochloric acid, add a few drops of calcium chloride solution and a few c.c. of chloroform with shaking. Bromine imparts a brown-yellow color to the chloroform. Bruce's test should also be available here. The yellow nitric acid test, as in the case of iodine, imparts a brown-yellow to the chloroform. Fresh chlorine water may be used instead of yellow nitric acid. Another way to detect bromine is to make 10-20 c.c. of urine alkaline with sodium carbonate, evaporate in a platinum crucible, char, extract residue with a few c.c. of distilled water, acidulate with hydrochloric acid, add a little chlorine water and shake with carbon disulphide which will be colored yellow. If iodine is present it will mask the bromine, which may be avoided by adding 1 c.c. of petroleum benzine to the disulphide extract decolorized by chlorine water and the iodine will then be extracted by the benzine.

Mineral Acids and Caustic Alkalies.—The urine reduces the copper test liquids but sugar is absent.

Nitric acid is detected by adding a solution of brucine to the urine, after which sulphuric acid is allowed to trickle down the side of the glass. At the point of contact a red ring is formed.

Urine may be tested for nitric acid also by allowing it to trickle down the side of a dish containing a concentrated solution of diphenylamine in sulphuric acid, which gives a blue color in the presence of nitric acid, nitrates, or nitrites. Normal urine, however, may contain traces of nitrates and nitrites.

DETECTION OF METALLIC SALTS.

Potassium Chlorate.—Acidulate the urine slightly with acetic acid, heat, and maintain for one minute at the boiling-point, filter, evaporate the filtrate to small bulk on the water-bath and allow to crystallize. The crystals are removed, dried on filter paper and tested. Treated with dilute hydrochloric acid and warmed, the fluid assumes a greenish-yellow color and gives off chlorine, recognized by its characteristic odor and suffocating fumes. Or the crystals may be dissolved in water and a solution of indigo and dilute sulphuric acid added. On further addition of aqueous sulphurous acid, the fluid changes from blue to yellow or loses its color entirely.

If no crystals form, evaporate the urine entirely to dryness before adding the indigo, etc.

Lithium.—Evaporate 100 c.c. of urine to dryness, and incinerate in a platinum crucible; add a little water and a couple of drops of hydrochloric acid; evaporate this solution. Extract the residue with strong alcohol; evaporate this. Dip a loop of platinum wire into it, or, better, its solution in pure hydrochloric acid, and bring before the slit of the spectroscope.

Arsenic.—A very large quantity of urine is to be evaporated to one-eighth its volume, about the same amount of pure hydrochloric acid, free from arsenic, added, and the mixture warmed on the water bath; chlorate of potassium is gradually introduced up to a few grammes, until the fluid is bright yellow, the free chlorine removed by evaporation, the fluid well diluted with water, and for several hours a stream of sulphuretted hydrogen allowed to pass through it. The precipitate of sulphide of arsenic is collected by filtering, washed, and dried; the filter is then put into a porcelain evaporating dish and a few drops of fuming nitric acid poured over it, then warmed on the water bath; con-

centrated sulphuric acid is added, and the fluid warmed until every trace of smell is gone. The fluid being then diluted with water can be tested by Marsh's or other test. (MacMunn.)

Lead and Copper.—Take at least one liter of urine in an evaporating dish, and evaporate to dryness over a water-bath. Add to this residue about 100 c.c. of pure concentrated nitric acid, and continue the heat until the acid has evaporated, when a yellow cake remains. Transfer this yellow mass—nitrates and nitro-compounds—to a crucible by means of a porcelain spatula, heat with a Bunsen flame until the mass ignites, and continue the heat until a white residue remains. Cool, add 10 to 20 c.c. of pure concentrated sulphuric acid, and heat until all of the nitric acid has been expelled—that is, until the red fumes disappear and dense white fumes are evolved. Cool, and then add from 25 to 50 c.c. of distilled water, and filter, reserving the filtrate for the test for arsenic. The precipitate on the filter is washed several times with distilled water in order to remove all soluble sulphates, and the final residue on the filter reserved for the test for lead.

Process for Lead.—The residue on the filter-paper, which consists of insoluble sulphates, including lead sulphate, is thoroughly extracted with hot dilute ammonium acetate containing an excess of acetic acid, and then filtered. A current of sulphuretted hydrogen is passed through the filtrate for about an hour, the lead acetate being precipitated as lead sulphide. Filter, dissolve the residue in hot dilute nitric acid, run into an evaporating dish, and evaporate to dryness over a water-bath. The residue in the dish is then dissolved in hot dilute acetic acid and filtered. The filtrate, which contains the lead in the form of an acetate, is then treated with either a few drops of a saturated solution of potassium bichromate, or a few cubic centimeters of dilute sulphuric acid and allowed to stand twenty-four hours.

The solution that contains the lead chromate or sulphate is then filtered and the precipitate, which is extremely slight, is washed a few times with distilled water. Sulphuretted hydrogen water, which has been previously filtered, is allowed to pass through the filter paper holding the precipitate and the filter then carefully dried. If lead be present, a slight black precipitate will be seen adhering to the surface of the filter paper near the center.

Process for Copper.—To detect copper salts proceed as above and test for copper by dissolving the sulphide of copper obtained in nitric acid with production of a blue color which becomes deeper on addition of ammonia. The nitric acid solution may also be tested with potassium ferrocyanide, yielding a red-brown precipitate.

Electrolytic Method for Lead.—This is claimed to be more reliable than the magnesium ribbon test. Add to 250 c.c. urine in a porcelain dish 2 c.c. of strong pure sulphuric acid, heat gently, add five grammes of potassium persulphate in small quantities with occasional stirring. Gradually bring to a boil until in half an hour the mixture is colorless. If it blackens, keep up the heat and add more acid (using a Kjeldahl flask) until decolorized. Heat until only 50 c.c. are left, then pour into a platinum dish and pass a current through it for eight hours from a couple of primary cells or from a storage battery. The platinum dish is the negative pole. Wash the dish with distilled water, add nitric acid to the washings, remove excess of acid by heat, and run in sulphuretted hydrogen into the liquid thus obtained. If lead is present a dark precipitate is obtained.

Another Method for Lead.—It is frequently required for clinical purposes in case of plumbism, to watch the gradual elimination of lead under treatment, especially after the administration of potassium iodide. As the amount of lead is often small, the whole twenty-four hours' urine should be employed. This should be evaporated to a thick residue in a large porcelain dish, and this transferred to a platinum or porcelain crucible, and heat applied, gently at first, till nothing but a gray ash is left. The soluble salts are then removed by the addition of boiling distilled water till a drop of the washings gives no residue when evaporated on a glass slide. The washings are to be examined with sulphuretted hydrogen to see that they contain no lead. The residue is then treated with equal parts of distilled water and nitric acid, and the mixture boiled for a few minutes; it is then filtered, and the filtrate tested for lead: (1) By the addition of sulphuretted hydrogen, giving a black sulphide of lead; (2) potassium iodide.

a yellow precipitate of lead iodide; (3) potassium chromate, a yellow precipitate of lead chromate, insoluble in dilute acids. These precipitates, together with the residue not dissolved by the nitric acid, and the precipitate given by the sulphydric acid, if any, with the first washings with hot distilled water, must be placed on a filter and dried. The dried residue is mixed with sodium carbonate, and the mixture placed on charcoal and fused by the blow-pipe, when a bead of metallic lead will be obtained, which represents the whole of the lead in metallic state in the twenty-four hours' urine. (Ralfe.)

Silver.—Evaporate the urine, destroy the organic matter by fusing the residue with nitrate of potassium and sodium hydrate, extract with water, and dissolve what is left over in nitric acid, filter the last solution, evaporate, dissolve in water and precipitate with hydrochloric acid. Chloride of silver is obtained, which is then tested in the usual manner.

Mercury.—A simple test for mercury in urine is that of Almen: to 300 c.c. of urine add a little sodium hydroxide, some cane-sugar and then boil the mixture. The phosphatic precipitate carries the mercury with it. Centrifuge or decant and dissolve the sediment in hydrochloric acid. Introduce a piece of fine copper wire and warm for an hour and a half. Remove the wire, boil in alkaline solution, and dry with filter paper. Place the wire in a glass tube of small caliber, break off a few millimeters in front of the wire, fuse at the end and heat over a small flame. The mercury sublimes and deposits in small globules recognized by the microscope.

Lombardo's Method for Mercury.—This is claimed to be more practical than those given above and is the following: into a centrifugal tube is poured 5 c.c. of filtered urine; one drop of egg-albumin is added and the whole shaken; after this 3 c.c. of a 12 per cent. filtered solution of tin chloride are added and the whole centrifuged several minutes. The precipitate under the microscope with a power of 600 diameters shows minute black globules of metallic mercury.

Iron.—In the freshly voided urine ammonium sulphide produces a greenish-black color.

DETECTION OF ORGANIC COMPOUNDS: ALKALOIDS.

Among the organic compounds found in urine are alkaloids, coal-tar compounds, resins, and a number of others.

Alkaloids.—Among those which may be found in urine are brucine, heroin, morphine, strychnine, atropine, nicotine, veratrine, cocaine, and quinine.

Morphine.—Evaporate the urine on the water-bath to a syrup and extract the residue several times with absolute alcohol; the united alcoholic extracts are evaporated, the residue extracted with water acidulated with a few drops of acetic acid, and the solution then repeatedly shaken with amyl alcohol warmed to 70° C. (168° F.) in a separatory funnel until the fluid is clear and colorless. The aqueous solution retains the alkaloids, while the urea goes over into the amyl alcohol solution.

To detect morphine the aqueous solution is made strongly alkaline with ammonia and shaken twice or thrice with hot amyl alcohol which on evaporation leaves morphine behind. To purify the residue it is dissolved in dilute acid and the treatment with amyl alcohol several times repeated.

To detect the morphine:

(1) Dissolve in strong sulphuric acid, add a drop of water, and a small fragment of dichromate of potassium. A mahogany color is produced.

(2) Heat the sulphuric acid solution in an air-bath at a temperature of over 100° C. (212° F.) up to 150° C. (302° F.) for ten minutes, allow to cool and then add nitric acid. A fine dark violet coloration is produced, which gradually becomes blood-red.

Morphine is also isolated by the Stas-Otto process as in the case of nicotine and atropine. The alkaline aqueous solution from which the ethereal extract has been removed is treated with ammonium chloride and repeatedly extracted with warm amyl alcohol. The amyl alcohol extract is next collected, filtered, and evaporated on the water-bath. The residue is dissolved in acidulated water which is repeatedly added for the purpose. It is then filtered, extracted with amyl alcohol, neutralized with ammonia, and again extracted with warm amyl alcohol, which is

again driven off by evaporation. The residue is then tested with Froehde's reagent and, if morphine is present, the fluid turns first violet, then blue and green, and finally pale red. The reagent must be freshly prepared by dissolving from 5 to 10 milligrammes of sodium molybdate in 1 c.c. of sulphuric acid.

Another test is with a solution of sublimed ferric chloride: dissolve a portion of the residue in water acidulated with hydrochloric acid. Evaporate to dryness on the water-bath and treat with a few drops of a very dilute solution of sublimed ferric chloride (free from acids) which, if morphine is present, gives the residue a blue color.

Another test is to drop the dilute solution of morphine into 1 c.c. of fuming (strong, pure) hydrochloric acid to which 1 drop of strong sulphuric acid has been added. Evaporate the mixture on a porcelain dish at 100°-120° C. (212°-248° F.) and a purple red color is formed. Take up the residue with strong hydrochloric acid, neutralize with sodium carbonate, and add to it a trace of an alcoholic iodine solution, when a brilliant yellow-green color is obtained which is soluble in ether to a purple.

Dionin and heroin also give morphine reactions, or at least some of them.

Brucine.—If the urine be evaporated, the residue should give a blood-red color with nitric acid or with sulphuric acid and potassium nitrate.

Colchicine.—The urine having been evaporated should give with strong nitric acid a violet color becoming finally brown-red or on addition of alkalies an orange-red.

Cocaine—This alkaloid is usually rapidly decomposed within the body. The urine residue should be heated with 2 or 3 c.c. of chlorine water and 2 or 3 drops of a 5 per cent. solution of palladium chloride added which, in the presence of cocaine, should yield a brilliant red precipitate. Crystals of cocaine permanganate may be obtained by adding a 1 per cent. permanganate solution, drop by drop, to a watery concentrated solution of cocaine hydrochloride. It is possible that in cocaine poisoning the drug may appear as a glycuronate in the urine.

Codeine.—It is said that when codeine is taken the urine gives a blue color with Obermayer's test for indican.

Nicotine may be extracted from urine as follows: the urine is digested in a flask with alcohol and tartaric acid on the water-bath, allowed to cool, and filtered; the alcohol is then evaporated on the water-bath at 60° C. (140° F.) only and the remaining aqueous solution filtered. The filtrate is evaporated to a sirup on the water-bath and the residue extracted with alcohol, added little by little, until a flocculent precipitate forms, and then in greater quantities until no further turbidity forms. The alcoholic solution is then filtered, the filtrate evaporated on the water-bath, and dissolved in a little water. The acid aqueous solution is next shaken with ether, rendered alkaline with potassium hydroxide, and again with ether. Nicotine and atropine are found in the ethereal extract, but morphine remains in the aqueous solution.

To detect nicotine evaporate the ether at low temperature on the water-bath and obtain a brown or yellowish mass which, if dissolved again in ether and tested with an ethereal solution of iodine, yields an oily substance from which Roussin's crystals, ruby-red needles, slowly separate.

Atropine is isolated in the same way as nicotine. After driving off the ethereal extract a little of the residue is dissolved in a few drops of fuming nitric acid (nitrous?) and evaporated on the water-bath; a colorless substance remains which, when allowed to cool and treated with alcoholic potassium hydroxide, yields first a violet and then a cherry red color.

Caffeine.—May be unchanged or transformed into xanthine.

Quinine.—Make 50 c.c. of urine alkaline with potassium hydroxide and shake five minutes with ether. Pipette off the ether, evaporate and dissolve the residue in water acidulated with a few drops of hydrochloric acid. Divide into two portions and note fluorescence when one portion is treated with a few drops of strong sulphuric acid, and an emerald green ring when the other is treated with fresh strong chlorine water and ammonia water. Warm the first portion, add a little tincture of iodine and green plates of metallic luster will be obtained.

Veratrine.—Concentrate the urine to a syrup, render alkaline with potassium hydroxide, agitate with chloroform and evaporate it to dryness on the water-bath; treat the residue with ether, remove the ether, and evaporate it to dryness. To a portion of the residue add a few drops of concentrated sulphuric acid, and heat on the water-bath, when a crimson color will be produced, if veratrine be present. Dissolve the remaining part of the residue in hydrochloric acid; this solution is colorless, when cold, dark red when warm.

Strychnine.—Concentrate the urine to a sirup, render strongly alkaline with potassium hydroxide, and agitate well with chloroform. Separate the chloroform and evaporate it to dryness on the water-bath; to the residue add strong sulphuric acid and heat on the water-bath for one hour, then neutralize with carbonate of sodium and render alkaline with potassium hydroxide; agitate again with chloroform; separate the chloroform and evaporate to dryness in a small porcelain dish on the water-bath; dissolve the residue in a few drops of sulphuric acid, then slowly move a small crystal of dichromate of potassium through this solution; if strychnine be present the crystal will produce a purple coloration.

COAL-TAR COMPOUNDS.

Under this heading will be considered anilin, antipyrin, antifebrin, carbolic acid, kairin, phenacetin, naphthalin, nitro-benzole, methylene blue, salicylic acid, and allied substances, pyramidon, thallin, etc.

Anilin.—The urine may reduce the copper test-liquids; the ethereal sulphates are increased notably and an ethereal extract may contain anilin shown by yielding a violet color in presence of a solution of so-called "chloride of lime."

Antifebrin.—Test for paramidophenol-sulphuric acid by boiling the urine with one-fourth its bulk of strong hydrochloric acid and, after cooling, add a few c.c. of a 3 per cent. solution of carbolic acid and a few drops of chromic acid. A red color appears, changed to blue by ammonia.

Another process is to shake the urine with chloroform, remove

chloroform, evaporate, and heat the residue with a little mercurous nitrate. An intense green color shows antifebrin.

Antipyrin.—The urine turns purple-red on addition of a few drops of 20 per cent. ferric chloride. This reaction is lost after the urine has stood a few days, is impaired by boiling the urine and destroyed by adding acids. If the urine be accidulated and extracted with ether a substance is obtained which gives a brown with ferric chloride.

Acetanilid.—This substance in urine yields the indophenol reaction like phenacetin, but if 2 drops of a 1 per cent. sodium nitrite solution be added to the urine with a few drops of sodium hydroxide solution and a little alkaline solution of alpha-naphthol a red color will appear which becomes violet with hydrochloric acid. Dark urines should be concentrated by evaporating, boiled with hydrochloric acid a few minutes, cooled, shaken with ether, and the residue dissolved in water.

Aristol.—Gives the iodine reaction in urine.

Asaprol in urine gives a blue color with ferric chloride.

Carbolic acid darkens the urine and will give a blue color with ferric chloride, but only after distillation with 5 per cent. of sulphuric acid.

Clinically, the presence of carbolic acid may be assumed when the voided urine assumes a dark green color or changes to black on standing and, at the same time, the preformed sulphates are diminished, shown by little or no precipitate when acidulated barium chloride solution (see Sulphates) is added to the urine, until after filtration and boiling with hydrochloric acid, when a copious precipitate occurs with the barium chloride. Alkapton and melanin both cause urine to darken on standing, but no green color is apparent and there is no marked alteration in the proportion of preformed and conjugate sulphates.

Moreover, the alkapton and melanin urines persistently contain the substances which cause darkening; in the case of alkapton there is practically no disturbance in the general health; in the case of melanin a tumor may be formed sooner or later.

Cryofin.—Same tests as phenacetin.

Creolin.—Causes a dark color and an increase in the conjugate sulphates.

Euphorin and Exalgin.—Give the indophenol test either directly or after distillation with potassium carbonate.

Exodin gives a dark color to urine, but the latter does not stain cloth. Alkalies do not change the color.

Lysol.—Increases the quantity of conjugate sulphates.

Eosin may be present in the urine of children who put the red end of red and blue pencils in their mouths. It gives a bright red color with alkalies, resembling red ink.

Dionin gives the morphine reaction.

Kairin.—The urine is greenish-brown, turns dark on standing, and deep violet or brown-red on addition of ferric chloride. Ether extracts it from the acidulated urine. Strong acids added to the urine or prolonged boiling impair the reaction. It is lævorotatory, reduces Fehling's on prolonged boiling, and is in glycuronic acid combination.

Lactophenin gives the indophenol reaction.

Methylene-blue colors the urine a blue-green. Color may be pronounced only after adding acetic acid and warming, or on standing exposed to the air.

The color is insoluble in ether, but may be extracted by shaking with chloroform and amylic alcohol. Urine containing it gives an absorption band between B and C and another between C and D.

Picric Acid.—Wool immersed in urine containing this acid is stained yellow after a long time. The urine warmed with potassium cyanide gives a red color.

Nitrobenzol.—The urine has an odor of bitter almonds, is usually lævorotatory and reduces the copper liquids.

Naphthalin is evcreted as alpha- and beta-naphthol glycuronic acid. If the urine containing it is floated on 2 c.c. of strong sulphuric acid, a brilliant dark green-yellow color is obtained due to alpha-naphtholglycuronic acid. If beta-naphthol is present in quantity a blue fluorescence will be given by addition of ammonia water.

Another test is to add to 5 c.c. of urine 3 or 4 drops of a

"chloride-of-lime" solution and the same amount of hydrochloric acid. A lemon-yellow color is obtained. Extract with ether, pour over a 1 per cent. watery solution of resorcin, shake with ammonia water, and a blue-green color forms changing to cherry red when nitric acid is added.

The urine darkens when naphthalin is taken as in carbolic acid poisoning.

Naphthol.—This is excreted as an ethereal sulphate. To detect it acidulate 500 c.c. of urine with hydrochloric acid, distill in steam, extract the distillate with ether and evaporate to dryness. Dissolve the residue in alcohol, add animal charcoal, gently warm, filter, and evaporate the filtrate. The residue should give a brilliant green-blue when warmed with a crystal of chloral hydrate.

Piperazine.—Urine containing it gives a precipitate with picric acid and with bismuth iodide dissolved in solution of potassium iodide. Filter off precipitated phosphates, acidulate the filtrate with hydrochloric acid, add the bismuth solution, warm, filter, cool, and get crystals.

Purgatin.—The urine is colored a Burgundy red and a white strip of linen soaked in it and dried becomes yellow.

Purgen (phenolphthalein). Is said to give the diazo reaction.

Resorcin.—This substance is excreted partly unchanged and partly as an ethereal sulphate. It answers to the naphthalin tests. Another test is to evaporate the urine to one-quarter its volume, boil the residue with sulphuric acid, extract with ether, evaporate the ether, boil the residue with barium carbonate solution, filter, treat the filtrate with animal charcoal, evaporate, dissolve the residue in water and obtain a violet color when ferric chloride solution is added, also a precipitate of tribromresorcin on addition of bromine water.

Phenacetin in urine reduces Fehling's solution on prolonged boiling, rotates to the left and gives the paramidophenol reaction like antifebrin. If large doses have been taken, the yellow urine gradually gives a brown-red or dark green color with ferric chloride or other oxidizing substances, changing slowly to black on standing for a long time. It gives the indophenol reaction.

Phenetidin may be tested for when the patient has taken phenacetin, as follows: to a little urine add 2 drops of strong hydrochloric acid and sodium nitrite (1 per cent.) solution. Then further add an aqueous alkaline solution of alpha-naphthol and a little sodium hydroxide which produces a beautiful red passing into violet if hydrochloric acid be added. It may be necessary to heat the urine first for a few minutes with one-fourth its volume of hydrochloric acid to split up ethereal sulphates.

Pyramidon.—A weak (2 per cent.) solution of ferric chloride added to the urine gives a dark-brown amethyst color.

Rosanilin.—Occurs in wines and may contain arsenic; detected in urine by the red color and by addition of ammonia followed by shaking with ether. Draw off the ether and introduce into it in a dish white wool, which is dyed red as the ether evaporates.

Salicylates.—These compounds, including also aspirin, show themselves in the urine by the occurrence of a dark purplish coloration, when a few drops of 20 per cent. ferric chloride solution is added to the urine. The color does not fade on standing. Salol and betal yield the same reaction with iron, as do salacetol, salipyrin, salophen, and salosantal. The salicylates are excreted partly unchanged, partly as salicylic acid, and partly as ethereal sulphates and glycuronates.

Thallin.—On adding ferric chloride the greenish-brown urine becomes purple-red, destroyed by boiling; or, if left standing, becomes brown-red in a few hours. Shake up with ether, remove the ether and add ferric chloride to it and obtain a green color.

Saccharin.—Passes out unchanged in the urine. Acidulate the urine with phosphoric acid, extract with ether and evaporate. The residue is sweetish to the taste.

Sulphonal, Trional, Tetronal.—These drugs manifest themselves by the presence of hematorporphyrin in the urine.

Urotropin.—When the patient takes this substance, it is said that with bromine water it gives an orange-red precipitate of dibromurotropin.

Resins, Etc.—☞ May give a whitish ring with the cold nitric acid test, soluble in alcohol. Turpentine may yield this reaction.

Copaiba.—Treat the urine with hydrochloric acid drop by drop and a red color appears, changing to violet when heated, hastened by addition of oxidizing agents. If ammonia water or sodium hydroxide be added to the urine, a light-brown color with a blue fluorescence appears. Boiled with acids a precipitate appears soluble in alcohol. The urine reduces Fehling's solution on prolonged boiling and is lævorotatory.

Sandalwood Oil.—Hydrochloric acid added drop by drop precipitates resinous acids of a reddish brown color.

Chrysophanic acid used in ointments and after use of rhubarb and senna gives the urine a brown-yellow color which turns purple-red on adding solution of sodium hydroxide.

Santonin.—Test as above, but shake with amyl alcohol, which becomes colored. The urine may be saffron-yellow or greenish, but on addition of sodium hydroxide becomes reddish, and the color is not extracted by ether.

Alcohol and Allied Substances.—Among these will be considered chloral and chloroform.

Alcohol.—The merest traces only can be found in the urine even in acute poisoning. The urine must be distilled with the steam-bath and the distillate tested with benzoyl-chloride and potassium hydroxide which, after heating and cooling, yields the odor of benzoylethyl ether; or cautiously mixed with an equal volume of strong sulphuric acid and heated with a little powdered sodium acetate the odor of acetic ether is obtained.

Chloral reduces the cupric solutions and is lævorotatory (urochloralic acid). In some cases it may yield a disagreeable odor when 10 c.c. of urine are heated with a drop of anilin oil and about 2 c.c. of alcoholic sodium hydroxide solution (isocyanphenyl test).

Chloroform reduces the cupric solutions and increases the quantity of chlorides. The urine is distilled by means of a steam-bath to prevent frothing and the first few drops of the distillate tested with Vitali's test, by which a dark violet tint is obtained when a little thymol dissolved in potassium hydroxide solution is added; or the isocyanphenyl test may be tried.

Miscellaneous.—Among other organic substances found accidentally in urine are *gallic* and *tannic* acids, as, *e. g.*, after administration of vegetable astringents, etc. They yield a bluish-black color when the urine is treated with ferric chloride solution. Gallic acid gives a green-black with ferric chloride; in alkaline urine a black slowly forming.

Carbonic Oxide (Carbon Monoxide).—In poisoning by this gas the urine invariably contains dextrose, recognized by the usual tests.

Cacodylic Acid.—Imparts an odor of cacodyl to the urine and if the latter be heated with phosphoric acid that of garlic. Arsenic may be found by the Marsh test.

Cantharidin.—The urine contains albumin, blood, and casts. Boiled with potassium hydroxide, decomposed with strong sulphuric acid and extracted with chloroform, the residue on evaporation dissolved in almond oil will blister the skin.

Guaiacol.—Add a little hydrochloric acid and distil the urine by conducting steam from another flask through the urine. To the distillate add dilute ammonia water and distil again. Dissolve the first portion of this distillate in equal parts of potassium hydroxide. An insoluble combination of guaiacol and potassium separates. Decompose with strong pure sulphuric acid, dissolve the guaiacol thus set free in alcohol and test with a trace of ferric chloride which gives a blue or a yellow-green if more be added.

Rhubarb.—Gives the urine a yellow color turned reddish purple by alkalies with formation of a red precipitate. The red color is not altered by acids being thus differentiated from the precipitate in Heller's test for blood.

Another test is to acidulate the urine with hydrochloric acid, shake with chloroform, and layer with solution of potassium hydroxide obtaining a violet ring.

Terpin Hydrate.—If 500 c.c. of urine containing this substance are distilled, the residue extracted with 5 c.c. of alcohol, the alcoholic extract heated and the vapor passed over a crystal of subchloride of antimony a red color will be imparted to it.

Thymol.—This substance is excreted partly as thymol-sulphuric acid, partly as thymol-glycuronic acid, and as a green coloring matter. If present in large amount, crystals of dichlorthymol-glycuronic acid may be obtained, when the urine is treated with one-third its volume of sulphuric acid and sodium hydrochlorite.

CHAPTER XXXIII.

LIFE INSURANCE EXAMINATION OF THE URINE.

Selection of specimen; time of day when the urine should be voided.
Physical characteristics of urine and meaning of them.
Albumin tests: relative value, technique, causes of error; Ogden's technique.
Sugar test: Haines' test, technique and criticisms, Fehling's test; washing out test-tubes.
Urea determination: instrument and technique.
The indican test.
Microscopical examination: search for tube-casts.
Significance of casts; corpuscles, and significance of them.

For life insurance purposes freshly voided urine which is passed in the presence of the examiner is a desideratum. In the case of females too much care can not be taken that no substitution is practiced. When the ordinary examination is doubtful, the entire 24 hours' urine should be demanded and comparison made.

The *time* of day when the urine is voided is of importance. The author prefers the *middle of the afternoon* when only one sample of urine can be had, and particularly dislikes to rely on the urine first voided on rising in the morning.

The *appearance* of the freshly voided urine is of importance. If the urine is cloudy when voided, it may contain pus or blood, which should be determined with care.

The *color* is also of importance, since too pale urine suggests diabetes and nephritis, and too red urine cardiac disease, cirrhosis of the liver, or other liver troubles; also pernicious anemia and drug addiction (sulphonal).

The *specific gravity* should be determined, since one below 1020 and especially below 1015 suggests nephritis; above 1025, diabetes mellitus if light colored, or renal and hepatic insufficiency if darker colored.

The *chemical reaction* to litmus paper should be taken, since a highly acid urine suggests diabetes mellitus, rheumatism, gout,

acute posterior urethritis and irritation of the kidneys—possibly, also, early stages of chronic contracting kidney—while alkaline urine is indicative of digestive weakness unless ammonia be the alkali, in which case bladder difficulties, spinal diseases, or a calculus may be the cause.

Next test the urine for **albumin and sugar** and remember that test-tubes are not clean until well washed and rinsed with *hot* water. Urine to be tested for albumin must be cleared by filtration.

The author does not recommend the use of the ferrocyanic test by examiners, unless they are experienced. The cold nitric acid test by contact is a good negative test. If it shows anything, the result should be confirmed by the heat tests with acetic acid and with salt. Remember that patients taking copaiba and sandal-wood oil may respond in their urine to these albumin tests, but that the resinous coagulum is soluble in alcohol.

Ogden claims that by underlaying urine with nitric acid rather than floating it upon the acid, three advantages result: first, a trace of albumin appears sooner; second, the zone of acid urates is widely apart from that of the albumin, and third, the **zone of balsams** lies **below** the juncture of the acid and the urine. Hence in doubtful cases this method of applying the test carried out by filling half full a perfectly clean, dry, and clear conical wine glass with filtered urine and underlaying with one-third volume of nitric acid is well worth considering.

☞ For sugar the Haines' test is the best negative test provided not more than 8 drops of urine are used with one fluidrachm of reagent. When the greenish-yellow cloudiness appears only after cooling, there is trouble in store for the examiner, who is advised in such a case to consult the author's scheme for detection of sugars. (See Chapter on Sugar.)

Ogden's claim that Haines' solution is not to be depended upon after one month, "although under some condition it may prove reliable for a longer time," is vague, and we think him mistaken. Why is it not to be depended upon and what are the conditions under which it may prove reliable?

Saxe's statement that "a worker of experience will prefer Fehling's solution" also suggests a similar interrogatory.

Both gentlemen are probably disturbed by the reddish deposit which takes place in Haines' solution at the end of a few weeks and which is of no consequence since the solution may be decanted or filtered free from it. However, if the company insists upon a test "made in Germany," Fehling's test should be used, but the author's criticisms of it in the Chapter on Sugar should be carefully studied.

☞ Instead of being obliged to make up Haines' solution fresh every month as suggested by Ogden, it is in the author's experience only necessary to decant the solution now and then in order to get rid of the reddish deposit; but in all cases test-tubes to be used with Haines' solution should be **washed out with hot water.**

Some companies require the determination of urea by the Doremus ureometer. If the percentage of urea is less than 1.5 collect the urine for 24 hours and if the total urea in 24 hours is found to be below 20 grammes (300 grains) it should be reported.

It is said that some companies now require the *indican* test to be made, which, if negative, suggests repeated trials, since by the author's or Askenstedt's method it is extremely unusual to obtain no color at all.

Inquiry should be made regarding acute attacks of any character affecting the urinary tract, such as renal colic, hematuria, or pyuria.

☞ When microscopical examination is to be made, look especially for *tube-casts.* The **transparent** hyaline casts, cylindroids, etc., are of doubtful significance, but the more prominent **glassy** hyaline casts and those containing cell elements or granules are significant. **The long, broad casts** are especially indicative of chronic lesions of the kidneys, as also any casts containing **dark coarse granules.** The number of casts and the presence of a variety of them should be observed.

Next in importance to casts are *red blood corpuscles* which require for identification considerable experience, since spores of fungi, present in nearly all acid urine soon after it is voided, may

be mistaken for them. Cleanliness on part of the examiner will help to rule out the latter.

Pus corpuscles (leukocytes) if present in appreciable number signify urinary disorder, and should be carefully observed if present.

CHAPTER XXXIV.

ESSENTIALS OF DIAGNOSIS BY THE URINE.

The history, clinical features and urinary findings in acute renal hyperemia.

Chronic renal hyperemia: relation to heart diseases. Symptoms and urine findings.

Febrile albuminuria (acute parenchymatous degeneration of the kidney): relation to acute infections; urine findings.

Acute nephritis: varieties, etiology, symptoms and urine findings.

Subacute nephritis: history, symptoms, urine findings.

Chronic nephritis: varieties, history, symptoms, and urine findings.

Amyloid disease: synonyms, history, symptoms, and urine findings.

Multilocular cystic kidney: general features.

Hypernephroma, malignant tumors: features.

Renal embolism: history, symptoms, urine findings.

Renal calculus: liability, symptoms, urine findings.

Hydronephrosis: history and urine findings.

Movable kidney: history, symptoms, urine.

Suppurative diseases of the urinary tract: renal tuberculosis, hematuria, pyuria, and symptoms.

Abscess of the kidney: history, symptoms, urine.

Pyonephrosis: general features.

Paranephric abscess: history and symptoms.

Pyelitis: history, symptoms, urine.

Ureteritis: general features.

Cystitis: causes, symptoms, urine.

Stone in the bladder: history, symptoms, urine.

Abscess of the bladder: general features.

Perivesical inflammation: features.

Tuberculosis of the bladder: history, symptoms, urine.

Tumors of the bladder: history, character of the hematuria, and of the urine.

The urine in benign growths.

Acute prostatitis: general features.

Urethritis (anterior): history, relation to gonorrhea; symptoms, condition of the urine.

Urethritis (posterior): two-glass test, etc.

Chronic urethritis: history and urine.

Chronic prostatitis: general features.

Prostatic abscess: pain, bleeding, and condition of the urine.
Tuberculosis of the prostate: features.
Hypertrophy of the prostate: general diagnosis.
Cancer of the prostate: diagnostic features.
Seminal vesiculitis: history and urine.
Spermatorrhea: features in the urine.
Acute infectious disorders: the urine and renal complications in general.
Scarlet fever: urine and the renal complications.
Smallpox: urinary features; albuminuria.
Meningitis: chlorides, albuminuria, glycosuria.
Acute articular rheumatism: renal changes, increase of uric acid, urinary signs of pericarditis.
Acute gout: physical character of the urine, uric acid features, occasional acute nephritis.
Typhoid fever: renal features, urine, etc.
Typhoid with nephritis: symptoms and urine.
Diphtheria: renal conditions.
Pneumonia: physical character of the urine, the sediment, urea, chlorides, albumin; acute nephritis in pneumonia; kind of casts present; pneumonia in chronic nephritis and in diabetes.
Erysipelas: renal complications.
Malarial fever: condition of the kidneys.
Yellow fever: urinary and renal features.
Cholera: the urine during collapse and after.
Bubonic plague: acute nephritis in severe cases.
Relapsing fever: renal features; glycosuria.
Typhus fever: urinary features.
Measles, whooping cough, infectious sore throat, influenza: features of the urine in these diseases.
Diseases of the blood: pernicious anemia, syphilis, and scurvy.
Gastrointestinal diseases: acute peritonitis.
Gastroenteritis, intestinal obstruction.
Appendicitis, cancer of the stomach.
Gastric ulcer.
Diseases of the liver: cirrhosis, functional disorders, jaundice, acute yellow atrophy.
The general urinary features in chronic diseases and those of Addison's disease, arteriosclerosis, chronic gout, pulmonary tuberculosis, diseases of the heart, diseases of the lungs.
Nervous diseases: the urinary features of hysteria, brain tumor, melancholia, nervous exhaustion, epilepsy, neurasthenia, acute myelitis.

Diabetes: the features in mild cases of diabetes mellitus and in severe ones. Diabetes insipidus.

Acute poisoning by external agents: the urine in poisoning by acids, alkalies, arsenic, alkaloids, alcohol, anilin, arsenetted hydrogen, carbolic acid, chloroform, copper salts, carbon monoxide, lead, mercury, nitrobenzole, phosphorus, ptomaines, sulphonal, etc.

Urinary findings in gynecological pathology: relation of pain and frequency to changes in the urine; "lithemia" and tumors; the nervous condition as shown by phosphoric acid; vesical irritability and high chloride content; estimation of total solids; significance of albuminuria and glycosuria; the indican reaction; pus in the urine of women.

The kidney complications of pregnancy: polyuria normal in pregnancy, decrease in urea common; cloudy urine of bad odor common; the diagnosis when albumin is found: essential albuminuria, the albuminuria of toxemia, and of nephritis.

Glycosuria during pregnancy: significance.

Diabetes in pregnancy.

Passive congestion, anemia of the kidneys, the kidney of pregnancy, pre-eclamptic conditions, acute nephritis, subacute nephritis, and pyelonephritis during pregnancy.

The following pages show the essentials of the history, symptoms and urinary findings in many diseases clinically common.

DISEASES OF THE KIDNEYS.

Acute Renal Hyperemia.—The history shows *physical strain,* as of athletic contests, *surgical operations* (with general anesthesia), especially on the urinary or genitourinary tract, or *acute poisoning* of external origin. The symptoms vary with the cause. If from over-exertion, the changes in the urine are the only signs; if from surgical operations, suppression of the urine and acute uremia; if from poisoning, frequency of urination, urgency, with perhaps pain and tenesmus. The urinary findings are diminution in volume, suppression of the urine, and more or less hematuria, with albuminuria corresponding. In the mild cases there may be but few red blood corpuscles seen microscopically.

Chronic Renal Hyperemia (Passive Congestion).—The history is usually one of valvular disease of the heart of long standing, of

cirrhosis of the liver, or of a tumor pressing upon the inferior vena cava. The symptoms are edema, ascites, hydrothorax, dyspnœa marked at night, rapid pulse, and weakness. The face is not swollen, the edema being mostly below the waist. The urine is scanty, the specific gravity normal or high, containing albumin in small amount, a few casts (yellow granular, hyaline, hyaloepithelial and finely granular) and often a sediment of urates.

Acute Parenchymatous Degeneration of the Kidneys and Febrile Albuminuria.—The history is usually one of an acute infection. The symptoms are those of the infection at its height and the urine findings may be merely deficient volume or a slight albuminuria, disappearing as the patient recovers from the infection. Cases occur in which either sepsis, anemia, jaundice, or poisoning from external agents may be the cause. In cases dependent upon jaundice the amount of the albumin is small, but bile-stained casts may be numerous. In septic cases the amount of albumin is small, but granular casts may be plenty.

In acute infections we find fewer casts, which are finely granular, yellowish or brownish-yellow granular, and hyaloepithelial or hyaline with a few red blood corpuscles.

Albumin is never large in amount in these cases, varying from a slight trace to possibly a slight deposit at the bottom of the Aufrecht tube, or a few per cent. by bulk in the Purdy tube.

The essential difference between acute parenchymatous degeneration and acute renal hyperemia is chiefly a pathological one rather than clinical, since in the former vascular phenomena are absent; hence blood is not found in the urine not even microscopically.

☞ **The Toxemia of Pregnancy.**—The history is that of primipara or of one who has had convulsions in a previous pregnancy, frequently a neurotic or of neurotic family history.

☞ The symptoms are suddenly appearing headache, edema, burning pain or pain in the epigastric region, more or less blindness or dizziness, nausea, vomiting, convulsions, and coma. The urine findings are increased color, diminished quantity, suddenly

appearing albumin in large amount without blood and with but few casts or none. The ratio of urea to ammonia may be low. The albumin may lessen in amount suddenly after convulsions or delivery.

Acute Nephritis may be either glomerular or interstitial. Acute diffuse glomerular, as after scarlet fever, has a history of sudden beginning after acute or chronic infection or acute poisoning of external origin, and is more common in children or young persons, especially after scarlet fever: the symptoms usually appear in the second or third week of an infection and include edema of the lids, general anasarca, waxy pallor, drowsiness, and uremic symptoms. The urinary findings are diminished volume, sometimes suppression, albuminuria with casts plenty, and often hematuria (beef-tea urine). Acute interstitial nephritis has the same history of infection, but comes earlier in the course and shows itself usually by an intense, but rapidly subsiding albuminuria without other features; rarely edema and uremia.

Subacute nephritis (subacute glomerular nephritis, chronic parenchymatous nephritis, large white kidney, fatty kidney, etc.) has a history of gradual development after infection or arises obscurely and insidiously from unknown causes, but more commonly in children or young persons than in the middle-aged or elderly; the symptoms are weakness, persistent anasarca, ascites, pallor, difficulty of breathing, gastro-intestinal symptoms, less commonly uremic ones until late. Dropsy is the characteristic clinical feature. The urine is highly albuminous and casts are numerous; blood may also be present.

Broad hyaline, dark granular, and fatty casts are characteristic. Free fat and fatty or granular masses may also be found and waxy casts occur. The disease may last several years. Sudden apparent recovery is not uncommon.

Chronic Nephritis (Chronic Interstitial Nephritis Including Primary and Secondary).—The history is of an unrecognizable invasion in primary cases, without previous renal disease, but in secondary cases it always follows subacute. The symptoms are cardio-vascular and uremic: hypertension, accentuation of the

aortic second sound, displacement of the apex beat to the left; headache; digestive symptoms, dizziness, attacks of unconsciousness, convulsions and coma; in the last stage edema beginning at the feet and chronic uremia.

The urine findings are polyuria or nocturnal polyuria, a pale color, diminished specific gravity, small amount of albumin and few casts in primary cases; more albumin, with more casts or fatty masses in secondary cases.

In the last stage of both forms albumin increases, the volume of urine diminishes without increase in color or specific gravity, and casts are more plenty.

In primary cases albumin and casts may be absent at times. The disease may be slow and last for years, but is liable to interruption from acute uremic attacks (convulsions, coma). Waxy casts may be found.

Amyloid Disease (Waxy or Lardaceous Kidney).—The history is one of chronic suppuration; the symptoms are chiefly gastro-intestinal with dropsy below the waist; the urine is clear and contains albumin, with globulin in relative abundance. Albumin may be enormous or small in amount. The sediment is scanty unless the condition complicates subacute nephritis. Hyaline and waxy casts are found in the scanty sediment. The disease is not likely to last more than a year and death is due to exhaustion or edema.

Multilocular Cystic Kidney.—The features are those of chronic primary interstitial nephritis with a soft non-fluctuant kidney-shaped bilateral renal tumor of slow growth. Blood may be found in the urine and in enormous quantity finally. The condition is rare in adults, as it is congenital and most of the cases die in early infancy.

Hypernephroma.—The history is that of a slow-growing tumor in persons 37 to 60 years of age, more common in men. The symptoms are pain of a dull character for some years, occasional hematuria, and slight cachexia. The urine, except when blood is present, is not characteristic.

Malignant Tumors.—The history is that of a renal tumor in

early or late adult life, more common in males; sarcoma more common in infancy or childhood. The tumor is limited in mobility. There is pain, pressure, progressive emaciation and cachexia, swelling, and unaccountable hematuria. Pressure on the inferior vena cava may cause dropsy of the lower extremities.

But for blood and large clots the urine may not be characteristic unless a coincident nephritis is present.

Renal Embolism.—History of endocarditis; sudden renal pain, perhaps with repeated chills and cardiac symptoms; if renal pain severe, vomiting and collapse. Sudden albuminuria gradually diminishing in two to four weeks; hyaline, epithelial, and leucocyte casts for a few days, then disappearing.

Renal Calculus.—Patient is likely to be younger than fifteen or older than fifty, of previous good health or "rheumatic" history, living in a lime-stone district, of sedentary habits, perhaps addicted to wine or beer drinking. The symptoms are a dull ache in the loin, or tenderness on deep pressure over one kidney, with attacks of renal colic and gastro-intestinal disturbances. The urine shows red blood corpuscles, especially after muscular exertion, more or less pus, and perhaps crystals. Sharp-pointed crystals of uric acid with pus and blood are characteristic, if found. Tube-casts are likely to occur (yellow-granular or hyaloepithelial), but not in great number.

Hydronephrosis.—The history is that of long continued obstruction to the free flow of urine and development of a tumor in the loin, whose size varies with the amount of urine. The urine may be normal or contain pus or blood. Sudden increase or decrease in the volume of 24 hours' urine with increase or decrease in the size of a tumor in the loin is significant. The patient is likely to be a woman.

Movable Kidney.—The history is that of a young woman, usually thin, who may have borne children in rapid succession or become emaciated for any reason. The symptoms are those of neurasthenia with renal pain or dragging sensation, movable tumor in the loin causing sinking sensation, or nausea when

handled. The urine is that of a neurasthenic woman; occasionally contains blood.

SUPPURATIVE DISEASES OF THE URINARY TRACT.

Renal Tuberculosis (tubercular pyelonephritis).—The condition is one of hematuria at night as well as by day in a person under forty with more or less temperature, and abundance of pus in the urine, especially occurring in puny, anemic males. There is more or less pain in the renal region with tenderness on deep pressure and later a tumor, constitutional symptoms, etc.

Suppurative Nephritis (abscess of the kidney).—The history is usually that of stone or prostatic disease and reveals also progressive loss of flesh and strength; the symptoms are nervous, digestive, and circulatory; the urine resembles that of chronic primary interstitial nephritis with pyuria. In acute cases, as, *e. g.*, following a chill in prostatic cases, sudden loss of strength, febrile disturbances, weakness, delirium and coma are seen. Pain or sensitiveness over one or both kidneys may be present. The rarely found bacterial casts in the urine are significant. Albumin and pus are abundant.

Pyelonephritis in Nurslings.—According to Lamalle, pyelonephritis is not a very frequent affection in nurslings. The greater frequency of pyelonephritis in little girls points to the urethra as the portal of infection. The main symptoms of pyelonephritis in nurslings are intermittent fever, accompanied sometimes by chills and sweats, and a turbid condition of the urine which continues after sedimentation has taken place. Freshly voided the urine exhibits an acid reaction; its microscopical examination shows the presence of leukocytes, a small number of epithelia, microorganisms, especially coli bacilli, and occasionally casts. Among the other symptoms may be enumerated edema, enlargement and sensitiveness of the kidney, and phenomena pointing to a mild uremic intoxication. The prognosis is comparatively favorable; in undernourished infants the prognosis is rather doubtful. (Abstract in Stern's *Archives of Diagnosis.*)

Pyonephrosis.—History of pyelitis or hydronephrosis, due most commonly to stone impacted in the renal pelvis. The tumor is

not so large as in hydronephrosis. Aspiration of the tumor shows pus. The symptoms and urine are as in pyelitis, but large amounts of free fat can sometimes be found in the urine.

Paranephric Abscess.—The history is that of a wound, surgical operation, suppurative lesion, as appendicitis, or pelvic cellulitis. The symptoms are fever, pain in the kidneys and finally swelling in the lumbar region. The urine is normal, unless the abscess ruptures into the urinary tract, when pus and fat suddenly appear.

Pyelitis.—The history is usually that of pregnancy, recent child-birth, calculus, tuberculosis, gonorrhea, or other infection. Symptoms may be absent; there may be a dull ache with sensitiveness on pressure over one or both kidneys and perhaps a slight temperature at night. The urine is likely to be acid and to contain pus, but symptoms during urination are wanting. Except in tubercular cases the amount of pus is small. Albumin is small and casts are absent. In old cases polyuria and pyuria with light-greenish feebly-acid urine, soon turning alkaline, is characteristic. The urine soon has an odor of rotten eggs, deposits triple phosphate crystals and shows pus corpuscles with tooth-like projections.

Acute Pyelitis Due to Bacillus Coli as It Occurs in Infancy.—The disease is commoner before than after the second year of life. It is more frequent in females than males, and more frequent in bottle-fed than breast-fed children. The cases are characterized by the extreme severity of their general symptoms and the trivial and equivocal nature of the local manifestations. The children are very ill, and yet there is nothing distinctive to be found beyond a little pus in the urine. The temperature runs up rapidly often reaching 104° F., or higher, and assumes a remittent type. Along with the sudden rise of temperature, there is, in a considerable proportion of cases, either a noticeable shiver or a definite well-marked rigor. The children are drowsy and often delirious. They are also restless, very miserable, and tender to touch all over. The blood shows some degree of leucocytosis. The local symptoms are either very slight or seem to

be quite absent. The characteristic features of the urine are the presence of a considerable number of pus cells and clumps of bacilli coli and a very distinct acid reaction. Three facts which are of importance clinically are: (1) at the time the temperature first rises pus is rarely, if ever, to be found in the urine; it always appears, however, in a few days; (2) the pus may at a later stage disappear from the urine for a day or two and then reappear; (3) although the urine is acid on passing, it tends rapidly to become alkaline on standing, and thus it is often difficult to distinguish pyelitis from cystitis. The diagnosis of pyelitis in children due to the bacillus coli rests on two things: the presence of pus in the urine along with the severe general symptoms just described and the absence of any ascertainable organic disease of other parts sufficient to account for these severe symptoms. Acute pyelitis is often mistaken for meningitis owing to the severe nervous and general symptoms which it occasions. (J. Thompson, *Quarterly Journal of Medicine,* 1910. Abstract in Stern's *Archives of Diagnosis.*)

According to Mirabeau vomiting setting in during the latter half of pregnancy is due to **pregnancy pyelitis**; even though but few leukocytes are to be found there is usually bacteriuria.

Ureteritis.—The history is usually that of some suppurative disease of the urinary tract, especially calculous pyelitis or of previous gonorrheal disease. The symptoms are not suggestive except in cases where there is an urgent desire to urinate followed by cramping pain ascending to the kidney (ureteral spasm). The finger in the vagina making pressure on the vesical end of the ureter may cause pain. The urine is that of pyelitis. Stone impacted in the ureter may be detected by the method of Heitzmann. (See Epithelium.)

Cystitis (Inflammation of the Bladder.)—*Causes:* Local bladder infection by bacterial germs: hence many causes, as gonorrhea, stricture, enlarged prostate, stone, sexual excess, etc.

Symptoms.—Pus in cloudy urine, frequent urination and pain, especially after urinating. No persistent constitutional symptoms.

The Urine.—In "acid cystitis" or more recent disease of the bladder the urine is acid when voided. More turbid in the first glass than in the second, plainly albuminous, with flocculent pus; microscopically, large round epithelia from middle layers of the bladder, pus corpuscles, blood corpuscles, and possibly bacteria, with a few imperfect crystals of triple phosphate. In "alkaline cystitis," or older cases, the color of the urine is lighter, reaction alkaline, odor ammoniacal or pungent or both; albumin in traces only, sticky pus; microscope always shows bacteria, chiefly the pathogenic, as *bacillus coli communis* and *staphylococcus pyogenes aureus,* pus corpuscles, blood corpuscles, bladder epithelia, and plenty of triple phosphate.

In cystitis the urine is always more or less cloudy when voided.

Stone in the Bladder.—The history is that of renal colic or of pain in the bladder, and disturbances of urination, especially in children or in old men with residual urine.

Symptoms.—The features are pain and interruption of micturition. The pain may be felt along the urethra, at end of penis, in testicles or down the thighs, is severe with spasm at close of micturition, and worse on motion; frequency of urination is present and is worse on motion.

Urine.—At first the urine is normal in appearance with deposit of crystals. Later the urine of cystitis appears plus crystals, and blood at the close of micturition aggravated by motion.

Abscess of the Bladder.—History, symptoms, and urine of cystitis, but the finger in the rectum shows localized induration, pain, and tenderness.

Prevesical Inflammation.—Shown by a sharply defined, usually symmetrical tumor just above the symphysis, sometimes suppurating.

Tuberculosis of the Bladder.—The history shows tuberculosis or cancer in the family, the patient is between 15 and 30, usually, and renal tuberculosis has been a primary condition. The symptoms are increased frequency of urination during the day, followed by hematuria and rising at night; severe tenesmus at close of micturition with constitutional symptoms of tuberculosis;

evening temperature, night sweats, etc. Features are relief from pain when the bladder is empty, persistent perineal pain, pain in the middle of the penis, hematuria without cause and not dependent on exercise.

The Urine.—Pyuria; hematuria, sometimes slight, sometimes pronounced; finally the urine of cystitis.

Tumors of the Bladder.—The history is usually that of a middle-aged male with an unaccountable hematuria. If the growth is benign, the symptoms are at first hematuria, then pain, frequency and relief of pain, etc., after a fresh attack of bleeding. The hematuria is intermittent with progressively increasing frequency. If the growth is malignant, the pain and the frequency precede the hematuria and continue without it.

The Urine in Cancer.—We find irregular and large shreds of connective tissue. Epithelia with very large, prominent nuclei; blood; features of cystitis. Epithelial nests in granular connective tissue are suspicious, and irregular shreds with nests characteristic.

Benign Growths.—We find typical shreds in the urine of connective tissue, of yellow-brown color like yellow casts. Great size is characteristic, and more regular form than in cancer.

Acute Prostatitis.—The history is that of gonorrhea, stricture, sexual excess or irritation from injections, instruments, bicycle riding, etc. The symptoms are constant, painful urination with feeling of something protruding into the rectum. The urine contains pus, blood, and albumin in small amount.

Chronic Prostatitis.—The history is that of acute prostatitis followed by symptoms of vesical calculus, but the amount of pus and blood in the urine is likely to be much less than in calculus.

Prostatic Abscess.—The history is that of acute prostatitis with chills, temperature, and lancinating pain. Blood may ooze from the urethra between urinations. The feeling of pressure is less great than in acute prostatitis. The urine contains pus and more or less blood.

Tuberculosis of the Prostate.—The history is that of debility or of tuberculosis slowly growing worse. The finger in the rectum

feels a lumpy prostate and a hard infiltrated vas deferens. The urine contains pus and perhaps, also, the bacillus tuberculosis.

Hypertrophy of the Prostate.—The history is that of an elderly man with difficulties of urination. The finger in the rectum feels a rounded dense mass. The urine is cloudy and contains pus, but albumin is small in amount and casts absent in most cases.

Cancer of the Prostate.—The history is that of prostatic hypertrophy with free hemorrhages from the urethra with or without urination. In some cases there are no urinary features. The prostate is hard and the pain almost uncontrollable even by opiates.

Seminal Vesiculitis.—The history is of gonorrheal urethritis. The urine of acute vesiculitis shows leukocytes, red blood corpuscles, vesicular epithelia, spermatozoa, vesicular shreds and plugs. In the chronic form there are also found the signs of chronic prostatitis and urethritis, masses of fatty epithelia from the vesicles, broken and distorted spermatozoa, plugs of coagulum, shreds of connective tissue, numerous round cells, mucous masses, and leukocytes.

Spermatorrhea.—Semen is found persistently in the urine, especially after stool, recognized by the presence of spermatozoa.

Urethritis (Anterior).—The history is usually that of a man who has been exposed to gonorrheal infection. The symptoms are free discharge of pus from the meatus, with or without urination, frequency of urination, painful urination and chordee. The urine shows a little pus and a trace of albumin.

Posterior Urethritis.—The history is that of anterior urethritis as above, followed by great frequency of urination with pain and voiding of acid urine. The urine voided into two glasses shows the second portion cloudy. Blood in small amount is voided with the last drops of urine.

Chronic Urethritis (Gonorrheal).—History of anterior and posterior urethritis not subsiding at the end of sixth week. Presence of filaments or slight discharge in the urine more abundant in the first glass than in the second.

THE URINE IN ACUTE DISORDERS.

☞ In many acute disorders the urine manifests certain characteristics which are merely indices of the condition of the kidneys and not specifically characteristic of the disease itself. If we take Ogden as a guide, in almost all acute infections the evidences of acute or active renal hyperemia are to be found, viz., diminished volume, increased specific gravity and color, acid reaction, albumin from a slight trace to a large trace, occasional or few granular or brown granular casts, some with blood and renal cells adherent, a few or numerous free renal epithelial cells and a few blood globules. On the other hand, the writer has examined the urine of many acute infections, and finds a number of cases in which red blood corpuscles are absent altogether, as well as blood from the casts, but leukocytes are present and granular casts not suggesting hemorrhage by color. Since edema is absent, as well as other renal symptoms, the author prefers the term "acute parenchymatous degeneration of the kidneys" to "active hyperemia," and refers the so-called "febrile albuminuria" to the irritation and degeneration of the renal epithelium by the toxins of infection rather than to renal congestion merely. Cases, however, undoubtedly do occur in which the latter condition exists, but in the author's experience they are by no means as common in infectious diseases as one would infer who reads Ogden and his followers. Fever urine, according to Ogden, is dependent on renal congestion, hence one should look for evidences of the latter in all acute febrile processes. But according to W. S. Carter in the section on General Pathology of Fever in the *American Text Book of Pathology*, the "Albuminuria seems to be due to changes in the kidney structure" and "occurs when the structural changes are most pronounced." These changes are "parenchymatous degeneration or cloudy swellings," etc.

Scarlet Fever.—During the height of the fever there is an acute renal hyperemia or a parenchymatous degeneration of the kidneys. The normal solids are not absolutely high. A true nephritis may, however, develop in the second or third week and pyelitis or pyelonephritis may also be present. The earlier the true

nephritis appears the more severe it is. (See Acute Nephritis.) The diazo reaction may be obtained in scarlet fever.

Chronic pyelitis or subacute nephritis may follow the acute pyelitis or acute nephritis. During convalescence from the nephritis polyuria may be present. A trace of albumin may be present for months but finally disappears.

Typhoid Fever.—In most cases during first week the features are those of acute parenchymatous degeneration of the kidneys (not nephritis), with high urea but low chlorides and phosphates. Urea may go as high as 60 to 70 grammes in 24 hours. Albumin as in acute parenchymatous degeneration, but in some cases as much as 0.1 per cent. by weight may be found. A few casts and blood, microscopically, may be present.

The diazo reaction occurs, after the fifth day especially. Pyelitis and cystitis occur as also urethritis.

Typhoid With Nephritis.—Occasionally true acute hemorrhagic nephritis occurs at the very beginning of typhoid or at the end of the first or second week. The symptoms are fever, backache, scanty, highly albuminous bloody urine with an abundance of casts. This condition is serious, but an acute nephritis during convalescence is more common and less serious.

Diphtheria.—The urine is usually that of acute renal hyperemia or of parenchymatous degeneration of the kidneys. In some cases acute nephritis appears, even quite early, but is less serious usually than that of scarlet fever. Occasionally the nephritis is fatal or is followed by subacute nephritis. Dropsy is not marked in the acute nephritis of diphtheria, but albumin may be enormous in amount. Sugar is sometimes found in the urine of diphtheria.

Lobular Pneumonia.—In general the urinary features are to be described as follows: The twenty-four hours' quantity of urine is greatly diminished, falling sometimes below 500 c.c. (one pint) in volume; the acidity is increased, the specific gravity increased, sometimes rising as high as 1040, the color is increased, and the odor more noticeable than in health. The normal solids are affected by the disease, urea, uric acid and phosphoric acid being increased both relatively and absolutely, the chlorides

diminished, sometimes absent, and the sulphates said to be diminished. Of abnormal constituents, albumin, albumoses, bile and blood may be present, and the diazo reaction may be obtained.

In the urinary sediment we find very commonly the brick-dust deposit of amorphous urates, also crystals of uric acid and calcium oxalate. Tube casts and renal epithelium occur under certain circumstances. Abnormal red blood corpuscles and leukocytes may be found.

Considering these various features in detail it may be said that the quantity of urine during convalescence increases from the low point as in other inflammatory diseases, the specific gravity decreasing along with the increase in quantity.

In regard to the acidity Pick claims that from 24 to 48 hours after the crisis the urine becomes alkaline or neutral and that the change from acid lasts from 24 to 36 hours, a point of prognostic importance if true.

The color and odor naturally decrease as the quantity of urine increases.

From a prognostic standpoint it may be said that a constant daily diminution of the quantity of the urine with increase in the specific gravity, color and acidity shows that the intensity of the disease is increasing; and conversely.

The quantity of urea present is of more or less importance in the diagnosis; in pneumonia daily averages of from 40 to 60 grammes, 620 to 930 grains, may be found, and there is record of a case in which 80 grammes, 1250 grains, were voided. The same is true of typhoid fever, but in other acute diseases we are not likely to find as much urea as in these two, hence knowledge of the quantity of it becomes of some value in the differential diagnosis from diseases other than typhoid.

In one case of pneumonia I found 40 grammes of urea in 24 hours, early, before the diagnosis could be definitely determined by the physical signs. In some cases characterized later by delayed convalescence, diarrhœal attacks, and other unfavorable conditions, the amount of urea is diminished during the first few days, hence a knowledge of the amount of the excretion becomes of some value from a prognostic standpoint.

After the crisis even when the urine is more abundant the amount of urea and uric acid may remain large, being perhaps formed from the exudate. The same is not the case in typhoid fever, so far as I am aware, where the urea diminishes in amount as the temperature falls.

The chlorides after the first day may be entirely absent for three days, reappearing as resolution occurs. The chlorides may be absent in other diseases attended by exudation, as pericarditis and meningitis, hence this phenomenon cannot be deemed characteristic of pneumonia.

In a case recently attended by Dr. Alfred Lewy, of Chicago, the patient was a child, female, six years of age, with pneumonia of the lower and middle lobes of the right lung, and extensive dry pleurisy of the right side. The crisis occurred on the twelfth day with a maximum temperature of 104° one day, after which time I found in the urine merely a trace of chlorides and the usual sediment of amorphous urates. Two days after the crisis the chlorides increased to six per cent. by bulk, and there was but a slight sediment of urates.

In regard to albumin, it may be said that at least traces of it are present in almost every case of primary acute pneumonia. The albuminuria is thought to be due, first, to irritation of the kidneys from the pneumotoxins; second, to congestion of the kidneys; third, to irritation from various unoxidized products, as uric acid and, perhaps, the xanthine bases. Lastly, in a certain number of cases the albuminuria may be due to secondary infection by the pneumococcus itself.

Occasionally, however, an acute glomerulo-nephritis complicates the pneumonia, due to pneumococcus infection of the kidneys; in such cases we find urine similar to that of post-scarlatinal nephritis, that is, containing much albumin, blood, numerous and various casts; there is a high fever, an irritable stomach and a clouded mind. The prognosis in such cases is unfavorable, and, if examination of the blood shows an active pneumococcus, the prognosis is hopeless.

Studying albuminuria in pneumonia with reference to prognosis, Sturges and Coupland found that in 27 cases in which it

was present in quantity, 5 proved fatal, and in 71 cases without albuminuria all but 2 recovered.

In regard to casts in cases not of pneumococcus infection my own experience has been that the granular variety is most common, of a yellow or brownish hue, and that the number of casts on a slide may be comparatively large even when but a trace of albumin is present.

It is true that pneumonia occurs in many cases toward the termination of chronic nephritis and of diabetes mellitus, but in such cases the clinical history and cardio-vascular changes of nephritis on the one hand and the presence of sugar in the urine on the other enable us to separate the previous chronic condition from the present acute disorder. It is needless to say that such cases usually terminate fatally.

OTHER INFECTIOUS DISEASES.

Erysipelas.—The usual features are those of acute renal hyperemia or parenchymatous degeneration, but an acute nephritis occurs in 5 per cent. of the cases and occasionally subacute nephritis occurs. Acute pyelitis may complicate the nephritis and chronic pyelitis follow it.

Erysipelas complicated with pneumonia, ulcerative endocarditis or sepsis is likely to be accompanied by severe acute nephritis and pyelitis resulting fatally.

Malarial Fever.—The features are those of acute parenchymatous degeneration during the pyrexia with polyuria after chill and fever. Reference has already been made (see Urea) to the increase in urea before the chill.

Acute nephritis is not common in malaria, occurring in less than 5 per cent. of the cases, but albuminuria is common, occurring in about half the cases. The nephritis is more common in the estivo-autumnal cases than in the regular intermittent cases. Contracted kidney is thought to be a frequent result of the nephritis of malaria.

Yellow Fever.—The features are those of acute parenchymatous degeneration of the kidneys in the milder cases, and the urea figure is an important element in the prognosis; the less the

urea in total the worse the case. In severe cases albumin appears on the first day and continues on the second, and a severe acute nephritis may be present. In some cases urea is totally absent. Bile is present in many cases.

Paroxysmal hemoglobinuria occurs in the malaria of the Southern States, and true hematuria of renal origin without much nephritis may occur.

Cholera.—During the collapse stage of the first two or three days the volume of urine is small or there may be complete suppression. The urea is low, being removed by the bowels, as are other normal solids. But the indican reaction is marked and there may be a sediment of indigo. Albumin, acetone and increased ammonia are found, as also increase in the ethereal sulphates. The albumin may disapper after the algid state is over, or if an acute nephritis is present during the algid stage, the usual convalescence from acute nephritis is seen after the algid stage is past. After the third day in favorable cases polyuria comes on and the amount of urea may run high for a few days (60-80 grammes).

Bubonic Plague.—Acute nephritis is not a common symptom but may occur in severe cases.

Relapsing Fever.—The usual features of acute hyperemia or acute parenchymatous degeneration may be observed. In severe cases acute nephritis is noticed, which, if hemorrhagic, is serious. Sugar is sometimes found in the urine.

Typhus Fever.—In milder cases the urine is that of acute renal hyperemia or of acute parenchymatous degeneration, and the chlorides are low or absent. In severe cases an acute nephritis may be present, as in typhoid, or hemoglobinuria may occur. At the crisis there is polyuria. Retention of urine is common.

Measles.—The urine is usually that of acute parenchymatous degeneration; nephritis is rare. The diazo reaction is common.

Whooping-cough, chicken-pox, infectious sore throat and influenza may be complicated by acute nephritis; dropsy with fatal uremia may occur. In influenza blood in the urine is rare. As a rule, the urinary features of these conditions are merely those of acute parenchymatous degeneration, but in whooping-

cough, according to Fuller, of Chicago, and others, a high specific gravity is common.

Smallpox.—The features are those of acute parenchymatous degeneration or of acute renal hyperemia in the ordinary form of this disease, but in malignant forms there may be a true acute nephritis. In the hemorrhagic cases hemoglobinuria is observed without nephritis. Leucine and tyrosine may appear in the urine instead of urea, but usually urea and uric acid are increased relatively and absolutely.

Albuminuria is always present whether with or without nephritis and usually at the onset with other features of acute renal hyperemia.

Meningitis.—Owing to exudation the chlorides are low or absent, and the acidity of the urine is diminished, hence earthy phosphates are thrown down on boiling the urine, thus differing from typhoid, in which chlorides are not so low and the reaction of the urine is acid. Albumin is always present, due to the acute parenchymatous degeneration or to acute renal hyperemia. In malignant cases a true nephritis may occur. Sugar has been found in the urine.

Acute Articular Rheumatism.—The usual features of acute renal hyperemia or acute parenchymatous degeneration are present. A feature of most cases is relative and absolute increase of uric acid.

A sudden fall in the amount of chlorides and phosphates betokens the advent of pericarditis.

Acute Arthritis (Gout).—During the attack the volume of urine is diminished, the color increased, and the specific gravity increased. Uric acid and phosphates are decreased during the paroxysm, but urea not much changed. The usual evidences of acute renal hyperemia are found in the trace of albumin, few yellow casts, etc. Between paroxysms, and especially just after the paroxysms, uric acid is increased and also other normal solids. A severe or fatal acute nephritis occasionally follows an acute gout.

Pericarditis.—(See "Acute Articular Rheumatism.)

DISEASES OF THE BLOOD.

In **pernicious anemia** the author has found a high color (urobilin) with a lowered specific gravity characteristic. A trace of albumin and a few yellowish casts may also be present. These features are more common during exacerbations. Uric acid is said to be increased and peptone to be present. In other forms of anemia—including at times in pernicious—the amount of urine per 24 hours is deficient, the specific gravity lowered and the various solids deficient. In leukemia the ratio of urea to uric acid is very low—as low as 15 to 1 is claimed—and the indican reaction is marked. A trace of albumin and a few casts may be present.

Syphilis.—Albumin in enormous quantity is sometimes a feature. Symptoms suggesting either acute or subacute nephritis may occur. Acute cases occur in two or three months after the chancre. The subacute cases are found in later stages. Cases of contracted, amyloid and gummatous kidney occur with the symptoms of chronic nephritis in general.

Scurvy.—The feature is hemoglobinuria, and this may be intermittent. Rust-colored stains are observed upon the diapers of infants affected by it. Albumin is therefore found, due to blood pigment, and a few casts may be present. Occasionally a true nephritis is present.

GASTROINTESTINAL DISORDERS.

Acute Peritonitis.—If the disorder is general, the chlorides are low and the indican reaction marked. Otherwise the features are those of acute parenchymatous degeneration or of acute renal hyperemia.

Gastroenteritis.—Children may suffer from a complicating acute nephritis. The symptoms are restlessness, persistent vomiting and cutaneous edema. The urine is that of an acute glomerular nephritis, usually of moderate severity.

Intestinal Obstruction.—In most cases the urine is that of renal congestion or of acute parenchymatous degeneration with scanty volume (due to vomiting and to the small amount of liquid taken) and a great excess of indican.

Appendicitis.—The volume of urine is diminished and a trace

of albumin may be found. The indican reaction is usually marked. There may be frequency of urination in the beginning of the attack, but no pus is found in the urine. Later, retention of urine may be present.

Cancer of the Stomach.—A significant feature in the urine is an increase in the ratio of urea to chlorides, *i. e.*, higher than 2 to 1, while at the same time the amount of urea is not excessive and febrile disorders are absent.

Gastric Ulcer.—The presence of acetone and albumoses is sometimes of help in the diagnosis.

DISEASES OF THE LIVER.

Cirrhosis.—The features are deficient volume, increased color due to urobilin, a trace or small quantity of albumin and a few casts, hyaline or yellowish granular. The peculiar reddish color is suggestive. The ratio of urea to ammonia is usually lessened in serious hepatic disorders. The benzaldehyde test or the zinc test may be positive. (See Urobilin.)

Functional Diseases of the Liver.—The occurrence of levulose in the urine after ingestion of it (alimentary levulosuria test) is regarded as a test of impaired function by some authorities.

Jaundice.—Bile in the urine is the feature, together with more or less acute parenchymatous degeneration of the kidneys due to irritation from the passage of bile; there is a plain trace of albumin, bile mucin, and often a large number of granular casts stained yellow by the bile.

Acute Yellow Atrophy.—The symptoms are jaundice and manifestations of brain lesion; the urine is both diminished and of low specific gravity while strongly acid. Urea may be absent or very small in amount, being replaced by leucine and tyrosine. Bile is present. Evidences of fatty degeneration of the kidneys are noticed., viz., much albumin, fatty casts, fatty epithelium and compound granular cells. But the elements are usually stained by the bile pigments.

CHRONIC DISEASES IN GENERAL.

The volume of urine in chronic diseases tends to be decreased, 1000 c.c. or below, the color is reduced, the reaction less acid and

the specific gravity not so high as in health. The solids are below normal, both relatively and absolutely. Chlorides are sensibly reduced. Traces of albumin may occur. The total phosphoric anhydride is noticeably reduced in most chronic diseases.

Addison's Disease.—A high ratio of urea to phosphoric acid when both are below the normal average in 24 hours' quantity has been noticed by the author. In one case this ratio was above 20 to 1.

Arteriosclerosis.—Long slender hyaline casts in the sediment, without albumin or with but a trace, have been found by the author.

In chronic gout chronic primary interstitial nephritis is common. Gouty glycosuria and oxaluria are common, and calculi may be found in the kidneys of gouty subjects. Occasionally the glycosuria merges into true diabetes mellitus.

Pulmonary Tuberculosis.—The urine tends to be strongly acid, and is said to remain acid for many days. If there is fever, the urine presents the usual features of fever urine. Uric acid is said to be increasd. Chlorides are much diminished if diarrhœa is present. When the lung tissues are rapidly breaking down, the phosphates are increased in total. Urea is lessened in amount unless the protein dietary is followed with success. In advanced cases the diazo reaction occurs and is of serious import. Albuminuria occurs also in advanced cases. Amyloid kidney and subacute glomerular nephritis are frequent complications. Pus may appear in the urine with blood, if the disease affect the urinary tract (tubercular pyelonephritis).

The diazo reaction is delayed in some cases. (See Diazo Reaction.)

Diseases of the Heart.—Mitral regurgitation and stenosis, myocardial disease and pericarditis may be marked by the general features of chronic (passive) renal hyperemia; so also obstruction or fatty degeneration of the right heart and aortic aneurism.

Diseases of the Lungs.—Emphysema, excessive pleuritic adhesions, or effusion, chronic interstitial pneumonia, chronic bronchitis and fibrous phthisis are all marked by the features of renal hyperemia.

NERVOUS DISEASES.

Hysteria.—The features are marked changes from normal in the volume of the 24 hours' urine, which may be totally suppressed for several days—eleven days possible—or after an attack enormously increased, as high as 5000 c.c., in 24 hours, of pale, watery urine.

Brain Tumor.—A case is on record in which tube-casts, including waxy, were found in the urine of a patient with a brain tumor.

Melancholia.—Patients with this disorder may refuse to drink, hence pass concentrated urine with abundant sediment of urates and calcium oxalate. The indican reaction is marked.

Nervous Exhaustion.—The feature is often a high ratio of urea to phosphoric acid—above 12 to 1.

Epilepsy.—After attacks there may be polyuria. Temporary albuminuria may occur.

Neurasthenia.—Neurasthenics pass urine abundantly, which is for the most part pale, of lowered specific gravity, deficient in solids and depositing almost no sediment. The night urine is copious, pale, and of low specific gravity.

Acute Myelitis.—Retention or incontinence of urine due to sphincteral disturbance is common, hence cystitis occurs with pyuria and hematuria, which may be followed by ascending pyelonephritis and coma. It is claimed that the indican reaction is marked in this disease.

DIABETES.

Diabetes Mellitus.—It is sometimes possible to detect this malady in the initial stages, which is shown by the appearance of sugar in small quantities at certain times of day, ☞ especially in the urine of digestion and more commonly two hours after the noonday meal.

☞ Mild cases are found in middle-aged, corpulent adults. The symptoms are polyuria, rising at night to urinate, more or less thirst, increased appetite, some loss of weight, more or less weakness and constipation. The urine is of high specific gravity and contains sugar. The acetone bodies are absent, and diet

causes the sugar to decrease materially or to disappear from the urine.

☞ Severe cases, on the other hand, occur in children or young persons, in those who have had the mild form for some years, and occasionally in adults without known previous history. The symptoms are as above, but are more marked. The patient wastes, grows weak, has gastric crises and goes into a coma after a few months or years. The urine contains acetone bodies and the sugar is not removed by the diet, usually remaining as high as 2 or 3 per cent. Even during a gastric crisis when, for several days, the patient is unable to retain anything on his stomach, the urine may contain sugar in considerable amount. The ratio of urea to ammonia may be lowered materially, 10 to 1 or lower.

Diabetes Insipidus.—The patient is usually a child or young person. This history is that of emotional disturbance, injury to brain, typhoid fever or prior infection.

The symptoms are great polyuria, great thirst, weakness and loss of flesh. The urine is of low specific gravity without presence of albumin, sugar or casts.

CARCINOMA.

According to Fuhs and Lintz the urine of cancer decolorizes methylene blue. If from three to five drops of Loeffler's methylene blue solution be added to a test tube full of urine, the mixture shaken and let stand twelve to twenty-four hours with a control tube of normal urine, the cancer urine will be decolorized. The same happens in other conditions besides cancer, as *e. g.*, pregnancy, but disappears as the patient improves, whilst in cancer it is persistent. The author finds that many samples of urine examined in all sorts of cases decolorize the blue, hence the necessity for repeated tests. Moreover, the test may fail in cancer.

URINARY FINDINGS IN GYNECOLOGICAL PATHOLOGY.

Frequency of urination and painful urination commonly occur in women, and a knowledge of the urinary constitution becomes necessary for a proper understanding of the pathology.

☞ Women may complain of scalding when a superficial ex-

amination of urine reveals nothing. In such cases, however, the collection of the entire 24 hours' quantity of urine, and a more complete analysis may throw considerable light on the annoying condition. First, what is the degree of acidity? Women with small or unduly irritable bladders are quick to complain when the degree of acidity of the urine rises sensibly above normal.

When the urine becomes concentrated the degree of acidity naturally rises in most cases, hence complaint of scalding should always lead to a collection and measurement of the 24 hours' urine, which, if found scanty, that is below 20 ounces and with a degree of acidity above 40, should suggest an increase of the amount of urine by suitable treatment. Sometimes the urine may be fairly copious, and yet scalding be complained of. In such cases it may be found that the ratio of acidity to total solids is high, that is though the amount of urea is low, and the specific gravity and total solids are low, yet the amount of acid is high in proportion. Such cases require treatment for any gastrointestinal or hepatic condition back of the undue acidity.

☞ Finally there may be cases of scalding in which repeated examinations of the 24 hours' urine in a thorough way fail to find the cause so far as the urine is concerned. Such cases suggest a purely gynecologic condition which is sometimes an early symptom in cancer of the uterus.

☞ Frequency of urination without pain is an annoying trouble to which many women are subject. In such cases the freshly voided urine should be examined microscopically for crystals since in some cases the deposit of crystals or even of amorphous materials within the body irritates the urinary tract and causes frequency. This is especially true of oxalate crystals and of the rarer sharp spiny crystals of uric acid. If the urine show these spiny uric acid crystals, the urine containing them is hyperacid even if the whole 24 hours' urine be normal in acidity. Hence in puzzling cases examination of the urine voided at different times in the day may be necessary since the condition of hyperacidity may be present only at times. It is possible also that certain micro-organisms not well identified may cause frequency; if the urine after being voided soon decomposes on exposure to

the air and the peculiar acrid odor of decomposing mucus be noticed, admixture with vaginal fluids being excluded, some such condition may be guessed at. The rare organism, leptothrix, in the bladder may also be a cause of frequency.

If the trouble is due to sharp spiny uric acid crystals, dilution of the urine and avoidance of alcoholic liquors and of hearty eating may help the condition; oxalate crystals may be lessened in number by a diet free from apples, bananas, rhubarb, tomatoes, sweets, coffee and tea. The phosphatic deposit may be cleared up by internal administration of acids and urinary antiseptics

☞ We must not overlook the fact, however, that frequency of urination in women is sometimes a symptom of grave constitutional disorders, especially at the menopause; nephritis or diabetes may then be the causative factor. Again, in stone and tuberculosis this frequency is often a feature. Hence the necessity for much care before pronouncing a case one of neurosis merely.

Acidity of the urine, while causing increased frequency of urination, also seems to aggravate almost any gynecological condition or at any rate it is common to find an increased urinary acidity at times of aggravation of gynecological conditions. The patient's comfort demands that we become aware of this hyperacid condition of the urine and mitigate it as much as we can.

Taking up the normal constituents of urine for consideration, one after another, we observe a low figure of urea in ovarian diseases and especially in ovarian cancer where as little as 6 to 8 grammes may be voided in 24 hours. Before operation, therefore, the estimation of urea should always be undertaken, and, if a low figure of urea be found, an attempt should be made to secure better elimination.

Since painful conditions in general are usually attended by diminished excretion of urea, comparative relief of pain before operation should be undertaken for the same reason as above. It has been noticed that in almost any morbid condition an aggravation of symptoms is accompanied by a decrease in urea and vice versa.

☞ It has been my own observation in two cases of abdomi-

nal tumors in women that a marked relative increase and also absolute increase in the amount of uric acid determined by the Folin method took place. For example in the case of a woman with an ovarian cyst, examined by me in 1906, the ratio of urea to uric acid was only 25 to 1, and the sediment contained amorphous urates and calcium oxalate. Strict diet failed to raise the ratio above 32 to 1. Just after operation the ratio was still only 27 to 1. Six weeks after operation after free use of French Vichy water the ratio rose to 42 to 1. It is well, therefore, not to assume that we have merely a case of "lithemia" in women when we find this low ratio, but to examine the abdomen, if the latter has not already been done.

Before operating for any gynecological condition an idea of the condition of the patient's nervous system may be derived from the quantitative determination of the total phosphoric acid in the urine of 24 hours. ☞ One of the most trustworthy signs of a depressed condition of the nervous system is a low figure of phosphoric acid compared with that of urea when urea is not in itself increased. Ratios of urea to phosphoric acid above 12 to 1 are found in nervous exhaustion. In Addison's disease, in which nervous prostration is most marked, I found in two cases ratios of 20 to 1 or higher. Ratios, therefore, from 15 to 1 and upwards are extremely significant of nervous exhaustion, and should arouse the attention of the surgeon to the fact that the cases demand immediate attention. I have looked up the mortality in nephritis and compared the analyses in the rapidly fatal cases with those pursuing a slow course, and have found that in 53 fatal cases analyses made by me not long before death showed an average excretion of phosphoric acid of only 1.14 grammes (18 grains) per 24 hours, and in 33 per cent. of the cases less than one gramme. In various nervous conditions supposedly reflex from uterine diseases, I have found 10 out of 13 to average less than 1.5 grammes of phosphoric acid. One woman with a fibroid, voided less than one gramme. If you wish to know how your patient is going to stand the anesthetic and the shock of operation make this analysis. I emphasize this statement because sufficient attention has not been paid to it. It stands to reason,

however, that repeated comparisons with the urea excretion must be made, for a single analysis, showing all normal solids to be decreased, might merely indicate low diet or some peculiarity of the diet on the day in question. If the patient's nervous system is exhausted, the ratio of urea to phosphoric acid will be higher than 12 to 1, and the higher the worse the condition. I have so many times verified this observation that I greatly desire to have the fact understood and appreciated in gynecological practice.

While speaking of phosphoric acid it may be worth while to explain that I am not making reference at all to phosphaturia or to a sediment of earthy phosphates in the urine. Such a sediment is claimed by some authors to occur in nervous prostration. But to my thinking it is not a trustworthy sign since alteration in the reaction of the urine from acid to alkaline from any cause will throw down the phosphates. The assumption that the sediment indicates nerve-waste is, of course, illogical and even absurd.

The determination of phosphoric acid as P_2O_5 is the desideratum clinically, and this is easily done by the method with uranium nitrate except in the case of ammoniacal urine. Normally the ratio of urea by the Doremus instrument to phosphoric acid by the uranium method is about 10 to 1, ranging from 8 to 1 up to 12 to 1 without much significance in this range.

So far as the chlorides are concerned a determination should certainly be made in cases of vesical irritability or scalding, and particularly in suppurative inflammations and ulcerations. No attention at all seems to be paid to the relation of the excretion of salt to irritation of the bladder except perhaps that in general the patient may be put on a milk diet. I examined the urine of a woman lately who was passing pus and blood, urinating every fifteen minutes in great pain. I found 12 grammes per liter of sodium chloride in her urine, fully a normal amount. Such a high figure of chlorides, relatively speaking, could hardly fail to irritate an already highly inflamed bladder.

The amount of sodium chloride voided by a patient on a mixed diet is of some value, prognostically, since an extremely low figure, 2 grammes in 24 hours, shows a grave condition, and ac-

cording to Guyon a fall in chlorides following operation below one gramme is a sign of impending death. In all chronic conditions a low figure of chlorides is construed as an unfavorable sign.

The estimation of total solids in the urine by the use of Haeser's co-efficient or that of Long, shows us a decreased amount of these solids in certain gynecological conditions. According to Delamater and Etheridge amenorrhea, neuralgia, pelvic peritonitis, backache and leucorrhea are conditions accompanied by a deficient excretion of total solids. When in any woman on a mixed det the figure of total solids falls below 20 grammes per 24 hours serious trouble may be looked for on taking cold. A close relation exists between pelvic disorders and renal insufficiency, and such a patient on taking cold may have an attack of peritonitis. If at the same time the acidity is high, aggravation of any pelvic condition is almost a certainty.

Albumin in the urine is a finding which deserves special study. In all cases in women where we think we find a trace of albumin confirmation of the finding by use of the salt test advised by Purdy and others is necessary. The urine of women quite commonly contains so much mucus and epithelium that with the ordinary heat and acetic acid test or the cold nitric by contact test a plain trace of protein is found, which, however, may be of no significance at all so far as the kidneys are concerned. The microscope will also show the absence of tube casts in verification. On the other hand, if we find a plain trace of this mucus-protein by the ordinary tests there is too much mucus in the urine and attention should be directed to the cause of the presence of it. Leucorrhea is a frequent source of so-called traces of albumin in the urine. Tamponing the vagina will serve to exclude admixture in the urine of vaginal fluids as already said.

☞ The finding of sugar in the urine of women is of significance in several ways; during the last weeks of pregnancy or during lactation a slight reaction for sugar with the copper tests may be merely referable to presence of lactose found in the case of women with superabundance of milk. The lactose does not ferment with yeast. When women complain of loss of sexual

desire or inability to experience the orgasm of coitus the urine should be tested for sugar, since in diabetes mellitus the sexual power is almost always seriously impaired. It goes without saying also that in cases of pruritus vulvæ, phlegmonous vulvitis and eczema of the vulva sugar may be at the bottom of the disorder. Especially is this the case at the time of the menopause. In young girls with amenorrhea the urine should be repeatedly tested for sugar since in diabetes the menses becomes scanty. The same is also true of nephritis.

Transitory glycosuria is reported in some cases of hysteria, hence in obscure cases in which the latter is suspected, the finding of such a urinary condition might be a link in the diagnostic chain.

Indigo-blue or indigo-red is a common finding in many gynecological cases and suggests either constipation or pus absorption. The diagnosis of the former is usually easy, but it must not be forgotten that fecal impaction is sometimes a difficult matter to be sure of and a persistent indican reaction in a woman's urine occurring when she asserts that the bowels move "every day" should arouse the suspicion of impaction and the size of the stools should be noticed.

☞ The indican reaction is, however, of the most importance to the gynecologist who suspects pus somewhere. Now there is a marked difference in the presence of indican due to pus absorption and that due merely to constipation or to toxemia from indigestion. In indicanuria from pus absorption five clinical points are to be observed: First, the reaction is brilliant, the amount of indigo-blue formed is large, the greatest amount is to be found in the night urine—another argument in favor of keeping the night urine separate from the day and examining it separately; meals do not affect the reaction, which is just as brilliant on rising in the morning or more so than at other times, while milk diet, alkaline waters, and bismuth salts fail to decrease the amount.

Coming finally to the consideration of the urinary findings so far as the sediment of urine is concerned, the first and most important constituent in the case of women is pus. Women are not as subject to kidney diseases as men, and when we find pus in the

sediment the last thing to think of is that it comes from the kidneys. The number of errors which have been made in practice by those who see kidney disease in every sediment of pus can hardly be estimated.

☞ Pus in the urine of women means vulvitis, vaginitis, urethritis, and cystitis more often than anything else. The diagnosis of pyelitis without confirmation by ureteral catheterization is an extremely risky one unless you have a history which suggests tuberculosis, or stone. Nohl mentions a case where a young girl was kept in bed for six months for supposed pyelitis when the condition was nothing but severe vulvitis. Hence, take every pains to exclude diseases of the genito-urinary tract before assuming that pus in acid urine always means pyelitis.

☞ Fistulous openings may make the diagnosis difficult. In one case in which I found pus in acid urine without any genito-urinary symptoms whatever and without discharge from the vagina, post-mortem showed that the pus focus was in the uterus, and in some way the pus drained into the bladder instead of out through the vagina.

Blood in the urine of women also suggests, first, the generative tract as its source, unless the tampon in the vagina or use of the catheter shows unmistakably the urinary origin. That the blood comes from the kidneys can be inferred by procedure, as follows: Procure a sample of urine freshly voided and examine it as soon as possible, say, within an hour. If it is acid in reaction and at the same time the blood corpuscles are small and blood shadows, *i. e.*, corpuscles from which the hemoglobin has been washed by urine, are present, appearing as rings in the field, the blood comes from the kidneys, especially when bladder symptoms are absent.

Women are subject to the rare condition of symptomless or essential hematuria. The blood is abundant and comes from the kidneys but there are no other clinical findings. Nephrotomy fails to find any cause but the hemorrhages cease after this operation. Later, the other kidney may bleed. Cases of this sort run in families, and in obscure conditions this fact may help in the diagnosis. In my experience it occurs in young women, just as essential albuminuria occurs in young men.

It must not be forgotten that strange and puzzling urinary findings occur in cases of girls or women who have introduced foreign bodies into the urethra, and which have slipped into the bladder and been retained there. It is difficult to obtain the history in such cases as the patients may be ashamed to confess the true cause of the trouble. By adopting the routine practice of having X-ray or cystoscopic examinations made in doubtful cases much uncertainty and trouble later may be avoided. I once examined the urine of a case of hairpin in the bladder and finding pus, blood and triple phosphate crystals in the fresh urine I naturally made a diagnosis of stone. As a matter of fact the hairpin when removed was incrusted with phosphates, thus serving as a nucleus for these substances. All history was negative in the case, and the patient even declared that she suffered no pain.

☞ In conclusion, I have only to caution those who have not been many years in the habit of examining urine, not to be too ready to assume the existence of chronic interstitial nephritis in women. The finding of a small amount of urea in the urine of delicate women who eat little meat, together with the erroneous assumption of the presence of a trace of albumin when only mucus protein is present, has led to many a diagnosis of chronic interstitial nephritis which was unwarranted. Kidney diseases in women do occur, but the various gastro-intestinal and gynecological conditions likely to be present make the diagnosis much less certain than in the case of men, and we need to exercise much care before committing ourselves to the opinion that nephritis is present.

THE KIDNEY COMPLICATIONS OF PREGNANCY.

Decrease of urea is a well known phenomenon of pregnancy, and is not necessarily in itself alone of pathological import. The authorities, and especially the more learned ones, refer the condition to hydremia and hypertension. It seems to me, however, that lack of exercise and restrictions of diet must have to do with it also. ☞ Whatever be the cause, the physician need not necessarily be apprehensive of uremia, because he finds only 10 to 12

grammes of urea in the 24 hours' urine of a pregnant patient. Other normal solids share in the decrease, namely, uric acid, creatinine, the phosphates, and the sulphates, which are said to pass over into the fetus. The chlorides alone hold their own or are even more or less increased unless the patient be put upon a milk diet, when they, too, will be greatly diminished in quantity. In general, then, the condition of the urine in a normal pregnancy is that of more or less polyuria, termed better hydruria, *i. e.*, increase in the water with decrease in the solids. The specific gravity is, therefore, quite regularly lower than normal. In some cases the polyuria becomes intense, simulating diabetes insipidus, with frequent voiding of much urine, and the patient suffers from thirst. But we are not advised by the authorities to take steps to lessen the polyuria which subsides after delivery.

Another thing noticed by many is the cloudiness and unpleasant odor so often present in the urine of pregnant patients.

☞This cloudy appearance of the urine, often coupled with an unpleasant odor, seems to be due to admixture of mucus in greater quantity than usual from the organs of generation and to bacterial change in it on exposure to the air.

In regard to abnormal constituents in the urine, the presence of albumin always excites more or less apprehension on part of the physician. This is aggravated in most cases by an unfortunate lack of previous history. The urine of girls is not examined as frequently as it ought to be. Now girls as well as boys are subject to *essential albuminuria,* a condition characterized by perhaps considerable albumin in the urine without other features either urinary or clinical. The condition is, as a rule, unnoticed and unsuspected, but during pregnancy it is likely to be discovered and to cause the attending physician no little worry. This is one of a number of reasons why I insist that the urine of all women should be thoroughly examined prior to marriage.

As a rule, however, if albumin is found early in the pregnancy without other urinary or general clinical features, essential albuminuria may be suspected. The albuminuria due to pregnancy itself is not likely to be noticed before the seventh month, excluding those traces of albumin which may be present in the urine

of any woman due to adventitious causes, *i. e.*, presence of epithelium and leukocytes in increased quantity from vaginal discharges, etc. ☞ Albumin not due to pus, blood, etc., in the urine of a pregnant woman in quantity sufficient to measure, when not due to essential albuminuria denotes anemia, congestion, toxemia or nephritis. If it steadily increases, to the last two rather than the first.

If the cold nitric acid test for albumin is positive and the boiling test negative albumoses are present, and death of the fetus is likely. In such cases acetone in the urine was formerly held as an important sign, but this has been lately disputed by Stoltz, who claims that acetonuria is merely a physiological manifestation explained by an alteration in the fat metabolism during pregnancy.

When the copper tests show sugar during pregnancy, care should be taken, if the patient is lactating, to ferment the urine in the Einhorn saccharimeter or similar instrument. If the sugar does not ferment it is lactose most likely, and certainly not glucose. But if it does ferment glucose is probably present. A small amount of glucose appearing only when the last few drops of urine are added to the boiling Haines' solution is held not to be of serious import in pregnancy. ☞ But diabetes in pregnancy with an appreciable amount of sugar is of a more or less serious character, decidedly serious if diabetes preceded pregnancy, and in any event an unfavorable condition sooner or later, though Duncan speaks of cases appearing during pregnancy and disappearing spontaneously after delivery. Such cases are probably rare, and I have never seen any. It is likely that they recur later. Most cases of diabetes are aggravated by pregnancy, though temporary improvement may set in after confinement. As a rule, the cases developing primarily commence in the last half.

The induction of premature labor in pregnant diabetics is, according to Lesse, only advisable in cases of hydramnion and contracted pelvis.

Diabetes may occur only during pregnancy and be absent at other times.

In some cases pregnancy is not affected by diabetes, but, as a rule, miscarriage occurs from death of the fetus.

Regarding the sediments found in the urine of healthy pregnant women, mention has already been made of the increase in mucus. Such mucus contains epithelium and leukocytes, and the amount of the latter increases. Using the centrifuge for sedimentation the microscopic field is likely to be full of leukocytes and vaginal epithelium in the latter months and during and after labor. Red blood corpuscles are not common until labor occurs. A few hyaline casts are not uncommon and need not be associated with albuminuria. When we find plenty of red blood corpuscles or numerous and various tube-casts the condition is likely to be pathological.

So much for the condition of the urine during pregnancy. Taking up the renal complications we find, first, *passive congestion* of the kidney supposed to be due to the pressure of the gravid uterus upon the inferior vena cava preventing the return of the blood from the kidneys. There may be those who have had more experience with this condition of pregnancy than I. The urine is decreased in volume, the specific gravity increased and the per cent. of the various solids, especially of urea and uric acid, should tend to increase, the urine is acid and deposits a clay colored sediment of amorphous urates. A small amount of albumin and a few casts, hyaline and yellow granular, may be found, and we should expect to see more or less edema about the feet. The chief importance of the condition is to distinguish it from the kidney of pregnancy and from acute or chronic nephritis. From the former it is distinguished by the small amount of albumin and absence of eclamptic symptoms, and from the two latter by the small amount of albumin, the increased specific gravity, small number of casts, the kind of casts, and the absence of red blood corpuscles.

It is argued, however, that since the increased intra-abdominal pressure of pregnancy affects the renal arteries as well as the veins the natural tendency is toward *anemia of the kidneys* rather than passive congestion. Inasmuch as the quantity of urine depends upon the blood pressure in the kidney, the prin-

cipal feature in anemia of the kidneys is decrease in the amount of urine. This gradual decrease in the quantity of urine is to my mind significant, since normally where pressure is not too great we expect to find a certain amount of polyuria in pregnancy. It has been noticed in some cases of eclampsia that the urine had been gradually decreasing in quantity for some days, and by some authorities anemia of the kidneys is thought to be the forerunner of eclampsia, since anemia leads to albuminuria from degenerative changes in the epithelium, and in chronic anemia, in general, fatty degeneration of renal epithelium is not uncommon. Hence a gradually lessening amount of urine in 24 hours, accompanied by albuminuria without the features of passive congestion mentioned above, is to be regarded as a sign of renal anemia and is a more serious condition than congestion from pressure merely.

☞ The so-called kidney of pregnancy is now regarded as a toxemic condition which is essentially a fatty degeneration or infiltration of the renal epithelium, varying much in degree, and which, with very few exceptions, undergoes complete repair after delivery, but which shows a curious tendency toward recurrence during subsequent pregnancies. In rapidly fatal cases the chief changes are in the epithelium of the distal portion of the proximal tubules. The kidney to the naked eye shows but little change, being only somewhat enlarged, pale, and of a greenish-yellow color. The condition is most common in the second half of pregnancy and in primiparæ.

Recovery from the kidney of pregnancy is the rule; in severe cases, however, it is said to lead to eclampsia, but the relation between the purely renal part of it and eclampsia is by no means certain since eclampsia may precede albuminuria, hence appear to cause the albuminuria. It seems more reasonable to reckon the kidney condition merely one of the various phenomena of eclampsia. ☞ Following a gradual diminution of the urine explained by anemia of the kidneys, a sudden decrease in quantity with increased specific gravity, high *percentage* of urea, and increase in quantity of albumin, which may become enormous in amount, signalizes the condition of eclampsia. Blood may not

be found in the urine of this condition before delivery, and tube-casts may be absent entirely in spite of the enormous amount of albumin. If present they are likely to be hyaline, hyaline with fat globules in them or finely granular.

A question of importance sometimes comes up as to the presence of a true *acute nephritis* in pregnancy. This is an entirely different condition, pathologically, from anything so far considered, but, clinically, the differentiation from the kidney of pregnancy is regarded by some authors as difficult. When we consider that a severe case of true acute nephritis may in any patient (as, *e. g.*, after scarlet fever) manifest uremic symptoms in the first few days the importance of the clinical history becomes manifest, especially as affecting our prognosis. The condition is probably rare, inasmuch as the acute infections are rare during pregnancy, and acute nephritis is dependent upon acute infections. In case acute nephritis occurs, the symptoms and urine are as in the non-pregnant, *i. e.*, we observe edema, waxy pallor and scanty, highly albuminous urine containing more or less blood and numerous casts, hyaline, yellow, leukocyte, blood, epithelial and finely granular. The finding of red blood corpuscles with the microscope should influence us toward the diagnosis of acute nephritis rather than pregnancy kidney; the diagnosis is of importance with reference to the prognosis which is held by some authorities to be less favorable than in eclampsia, since it tends to cause uremia and does not necessarily terminate with delivery but may become subacute and eventually chonic.

Subacute nephritis (which in the books is still called chronic parenchymatous) is by no means infrequent in pregnancy, and in my experience is the commonest of all so-called kidney complications. It is not caused apparently by pregnancy but exists in the patient before conception, and is roused to pernicious activity by pregnancy. ☞ Many female children have acute kidney trouble due to acute infections, and it is fair to suppose that some of the cases persist as mild subacute forms possibly unknown to the person. The girl marries, becomes pregnant, and sooner or later edema appears, the urine becomes albuminous, the amount of albumin gradually increases until it may be enormous in

quantity, and numerous casts are found which are usually large, broad, hyaline or dark granular or fatty. Yellow casts, red-brown casts and blood casts may be present. Such cases practically never have eclampsia and probably few have even uremic convulsions. But eventually something almost always happens. Premature delivery is common. If the labor is severe and complicated by operative intervention, the mother may die of uremia. The confinement may be normal, but sepsis follow even slight traumata. A severe acute exacerbation of the nephritis may set in during the lying-in period. Sudden collapse from heart failure may take place during the puerperium. Hence it is that most authorities insist upon the advisability of interrupting pregnancy in cases of this kind, just as they insist upon the advisability of early operation in all cases of appendicitis. Yet it has been my good fortune to help steer many a pregnant woman through the shoals and rocks of subacute nephritis to normal delivery, and recovery with a healthy child, hence I seldom advise interference. The mildest cases are the hemorrhagic ones, and in such cases I regard interference as probably unnecessary if proper care be taken of the case. There is this, however, about it. Subacute nephritis tends to become worse during successive pregnancies, and this fact has a bearing upon the advisability of interference a second or third time.

Floating kidney and tumors of the kidney seldom complicate pregnancy and labor.

Pyelonephritis has of late years been recognized as not a rare complication of pregnancy. It is said to be due to the colon bacillus gaining access to the urinary tract by direct propagation through the intestines. The condition appears any time after the fourth month, and is shown by severe constitutional symptoms (chill, fever, malaise) together with albuminuria and pyuria. Bladder symptoms must be absent to make the diagnosis. The condition clears up after labor, but may recur in successive pregnancies. The usual treatment of rest, milk diet, and purgatives is recommended together with intestinal antiseptics. I have not myself seen any cases of it, hence can claim no results of treatment.

Hydronephrosis is a kidney complication of pregnancy occurring as a result of pressure on the ureters by a uterus bound down by adhesions or by twisting of the pedicle of a dislocated kidney. The uterus or kidney must be replaced and held in position if possible.

Interruption of pregnancy usually occurs. (Edgar.)

ACUTE POISONING BY EXTERNAL AGENTS.

In poisoning by **irritants**, as strong acids, metals, etc., as a rule, the volume of urine is diminished, it is of high specific gravity and of acid reaction.

Acids.—Cause an acute toxic nephritis with blood and albumin in the urine. The urine also reduces the alkaline copper tests but sugar is absent.

Alkalies.—Albuminuria is common even in slight cases and evidences of nephritis present. The urine reduces Fehling's solution but sugar is absent.

Arsenic.—The urine contains albumin and sometimes blood in considerable amount. The urine reduces Fehling's liquid but sugar is absent. Occasionally the symptoms of acute nephritis supervene.

Alkaloids.—In acute morphine poisoning the urine contains sugar, and in chronic poisoning it sometimes reduces the cupric tests.

Alcohol.—Chronic poisoning is said to be conducive to arteriosclerosis and chronic nephritis.

Anilin.—The urine is usually dark and concentrated; it reduces the copper test liquids, but sugar is absent; the ethereal sulphates are increased. Workers in dyes are subject to cancer of the bladder.

Arsenetted Hydrogen.—Fatty degeneration of the kidneys with hemoglobinuria. (See "Phosphorus.")

Carbolic Acid.—The features are scanty urine, darkening on exposure to air. The ethereal sulphates are increased in amount. Renal congestion or acute nephritis may be present.

Chloroform.—The urine is of high specific gravity, may contain a trace of albumin and the glycuronate reaction may occur. The chlorides are increased.

Copper.—The volume of urine is reduced while albumin usually and sometimes blood appears.

Carbonic Oxide (Carbon Monoxide).—The urine always contains grape-sugar and an uncertain amount of albumin. (Jaksch.)

Lead.—In acute poisoning, especially when lead colic is present, large amounts of albumin appear for a time in the urine. In some cases nephritis is observed with edema, etc.

Mercury.—In a very few hours albumin in large quantity appears in the urine and often blood. Sooner or later edema and other symptoms of nephritis appear.

Nitrobenzole.—The odor of oil of bitter almonds is observed in the urine, which is lævo-rotatory and reduces the alkaline cupric solutions.

Ptomaines.—A case of acute nephritis is on record following sausage poisoning.

Phosphorus.—Fatty degeneration of the kidneys with hemoglobinuria. Urea low, leucine and tyrosine present. Albuminuria marked, granular and fatty casts and usually a little blood present. Fat has been found in large quantities. Bile, sarcolactic acid and fatty acids may be found.

Sulphonal, Trional, Etc., Poisoning.—Hematoporphyrinuria is a feature.

CHAPTER XXXV.

THE SYSTEMATIC CLINICAL EXAMINATION OF URINE AND THE LABORATORY EQUIPMENT NECESSARY FOR IT.

The collection of urine for examination.

Physical analysis.

Chemical quantitative analysis.

Sedimentation with the centrifuge.

Chemical quantitative analysis.

Card index report of analysis.

Report for hospital analysis.

Special analysis necessary in some 30 different diseases.

Clinical notes on various findings.

Apparatus and reagents: kind and number necessary of graduates, jars, cylinders, flasks, beakers, burettes, bottles, fruit-jars, pipettes, funnels and wash-bottles.

Special apparatus desirable: saccharimeters, ureometers, urinometers, albuminimeters, centrifugal tubes, special glassware, water-baths, tripods, drying-oven, Bunsen burners, burette-stands and clamps, alcohol lamps, distilling apparatus, casseroles, separatory funnels, Sohxlet extraction apparatus, Kjeldahl apparatus, polariscope, centrifuge, platinum ware, dishes, microscope, objectives, test-tubes, filters, etc., etc.

REAGENTS: solid and liquid.

Reagents in solid form: acids, salts, and organic compounds used.

Liquid reagents in large supply: names, amount desirable and methods of obtaining or preparing liquids used: acetic acid, ammonium compounds, chloroform, Folin-Shaffer reagent, Haines' test liquid, mineral acids, 25 per cent. nitric acid, potassium and sodium solutions, uranium nitrate.

Liquid reagents in smaller quantity: alcohols, dilute acetic acid, barium solutions, Barfoed's solution, benzoyl chloride, benzaldehyde solution, Bial's reagent, bromine, "chloride of lime," copper salts, diazo reagent, ether, Esbach's solution, Aufrecht's solution, ferric chloride, ferric alum, Fehling's solution, formaldehyde, guaiac tincture, glycerine, hydrogen dioxide, iodine solutions, lead acetate solutions, Lipliawsky's reagent, magnesia mixture, Millon's reagent, Moreigne's reagent, Nylander's solution, Obermayer's solution, phenolphthalein indicator, Purdy-Haines' solution, oxalic acid decinormal, various potassium so-

lutions, sodium chloride, sodium hydroxide decinormal, Sewilanoff's reagent, silver nitrate volumetric solutions, zinc acetate solution for urobilin, etc., etc.
Thermometric equivalents.

The urine having been collected, according to directions, for the whole 24 hours, with day, night, and fresh samples separately, the fresh sample is first examined, chemically, for albumin, and sugar; the sediment with the microscope after centrifuging. The day urine and night urine are next measured, each in a separate graduate glass and the volumes recorded. The physical characteristics of day and night urine are then separately noted and recorded. Chemical tests for albumin, sugar, bile, urobilin, acetone, diacetic acid, and indican are then made, the day and night being separately examined. While the urine is being filtered for the albumin tests, a sample each, of the day and of the night, is centrifuged and examined with the microscope.

After the qualitative tests and the microscopic examinations have been made of the day and of the night separately, then and not until then the two samples are mixed thoroughly and the quantitative analysis for total solids, acidity, urea, uric acid, phosphoric acid, chlorides, albumin, sugar and any other quantitative tests necessary made.

CLINICAL REPORT OF ANALYSIS.

The author finds it convenient to issue a report of the following character, printed on card paper of the size suitable for the card index system, and with two circular perforations. A synopsis of the findings should precede the detailed reports of the analysis.

REPORT OF EXAMINATION OF URINE: PHYSICAL, CHEMICAL AND MICROSCOPICAL.

N. B.—Before sending urine please get printed directions for collection and forwarding in order to obtain a more thorough analysis. Case number ..... Name ..... Report to .....

SUMMARY OF THE MOST IMPORTANT FINDINGS.

Abnormal Physical Findings: .....
.....
Abnormal Chemical Findings: .....
.....

Abnormal Microscopical Findings: .....
.....
Organs of the Body Apparently Diseased: .....
.....

THE ANALYSIS IN FULL.

A. Physical Analysis:

1. Volume of urine received ..... c.c., representing .....
2. Volume of urine per 24 hours .....c.c., day .....c.c., night .....c.c.

(The normal volume per 24 hours ranges from 900 c.c. to 1500; in children from 300 to 900. The ratio of day urine to night from 2 to 1 up to 4 to 1 or more if much liquid is drunk during the day.)

3. Color ..... (Normal is yellow).
4. Odor ..... (Normal is slight but agreeable).
5. Appearance .....(Normal is almost clear); sediment ..... (Normal is slight).
6. Reaction to litmus paper .....(Normal is slightly acid).
7. Specific Gravity by Urinometer .....(Normal range from 1018 to 1025).
8. Miscellaneous physical findings: .....

B. Chemical Analysis: Physiological Constituents:

1. Determination of acidity .....degrees, total in terms of HCl ..... grammes.

(Normal is 30 to 40 degrees, total 1.32 to 2.3 grammes HCl.)

2. Total solids, Haeser's or Long's coefficient ..... grammes (Normal range varies by age, weight, etc.; 63 grammes for those 20 to 40 years old weighing about 150 pounds).
   Relation to normal in this case .....
3. Nitrogen per 24 hours ..... grammes (Normal 10 to 15 grammes).
4. Urea per cent. ..... Per 24 hours ..... grammes (Normal 20 to 30).
5. Uric acid per cent. ..... Per 24 hours ..... grammes (Normal 0.4 to 0.6).
6. Purine bases per cent. .....Per 24 hours .....grammes (Normal 0.05 to 0.09).
7. Ammonia per cent. ..... Per 24 hours ..... grammes (Normal average 0.7).
8. Other nitrogenous constituents .....
9. Chlorides, as NaCl, per cent. ..... Per 24 hours ..... grammes (Normal 10 to 15).
10. Phosphates, as P_2O_5 per cent. ..... Per 24 hours ..... grammes (Normal 1.5 to 2.5).
11. Phosphates, earthy ..... grammes, alkaline ..... grammes (Normal 1.25 and 3).
12. Sulphates, mineral, per cent. ..... Per 24 hours ..... grammes.

(Normal 3 to 4 grammes): ethereal, per cent. ..... Per 24 hours ..... grammes. (Normal 0.3 to 0.4.)

13. Ethereal sulphates separately: Indican ..... Others .....
14. Neutral sulphur compounds ..... Total sulphur .....

15. Normal pigments and chromogens: urobilin, uroerythrin, etc. .....

16. Miscellaneous physiological constituents, oxalic acid, etc. ....

C. Ratios and Coefficients of Physiological Constituents:

1. Ratio of urea to total salts .....to 1 (Normal 0.75 to 1). 2. Urea to uric acid ..... to 1 (Normal 40 to 1). 3. Urea to ammonia ..... to 1 (Normal 35 to 1). 4. Urea to P_2O_5 ..... to 1 (Normal from 9 to 12 to 1). 5. Urea to chlorides ..... to 1 (Normal around 2 to 1). 6. Mineral to ethereal sulphates ..... to 1 (Normal 10 to 1). 7. Miscellaneous coefficients (nitrogen, ammonia, Baumann's, Combe's, etc.) ....

D. Chemical Analysis: Pathological Constituents:

1. Albumin, per cent. bulk ....., weight ....., remarks ....
2. Other proteins .....
3. Sugar (dextrose) per cent. ....., remarks .....
4. Other carbohydrates or reducing bodies .....
5. Acetone in quantity ..... diacetic acid .....
6. Pigments: blood, bile, etc. .....
7. Miscellaneous pathological constituents: .....
8. Special reactions: ferric chloride for drugs, etc. ....., diazo for typhoid, etc. ....., Rosenbach's for intestinal indigestion ..... Ehrlich's benzaldehyde for hepatic diseases .....
9. Tests for accidental constituents, drugs, poisons, dyes, etc. ....

THE URINARY SEDIMENT.

E. Chemical Analysis:

1. Amorphous constituents .....
2. Crystalline constituents .....
3. Pus, blood, etc. ..... 4. Calculi .....

F. Microscopical Analysis:

1. Tube casts .....
2. Crystals ..... 3. Corpuscles .....
4. Epithelium ..... 5. Miscellaneous .....

DETERMINATION OF THE RENAL FUNCTION.

G. Cryoscopy: .....

H. Tests for Insuffiency: (a) Indigo-Carmine .....
(b) Phenolsulphonephthalein .....

I. Tests for Permeability, etc.: .....

Signature .....**M. D.**

HOSPITAL ANALYSIS.

The above chart may be utilized for hospital analysis by omitting B. 3, 5, 6, 7, 8, 9, 10 to 16 except indican inclusive; also C., D., 7, 8, 9, and G.

SPECIAL ANALYSIS.

When any of the conditions mentioned below is suspected, test according to the following outlines:

APPARATUS AND REAGENTS.

The kind and quantity of apparatus used for urine analysis depends upon the kind and quantity of work done. The following is probably a list of the maximum requirements for clinical work:

Graduates.—These should be of the kind described under Physical Characteristics, *i. e.*, flat bottomed without feet. Provide 3 of 1000 c.c. capacity, 4 of 500 c.c., 2 of 250 c.c., 2 of 120 c.c., 2 of 60 c.c., and 1 of 10 c.c. In addition it is well to have one of the older style with a foot and holding 2000 c.c., or if this can not be had one graduated in ounces up to 64 fluidounces. One or two 60 minim graduates should also be kept on hand. ☞ Get them of Schmidt & Ade, Chicago.

Jars.—Large glass jars made of thick glass are useful for measuring the 24 hours' urine of diabetics. Those holding about 4000 c.c. or a gallon are desirable. Large thin glass jars should not be purchased as they break easily.

Cylinders.—At least one each of 1000 c.c. capacity, 100 c.c. and 50 c.c. should be owned. In addition, half a dozen small cylinders of 10 c.c. and 5 c.c. each will prove to be very serviceable.

Flasks.—Flasks are of two kinds, graduated and ungraduated. Graduated flasks are of fairly thick glass and have long slender necks with a graduation mark on the neck. It is desirable to use graduated flasks having the graduation mark high up on the neck where it can readily be seen in filling the flask. Provide 6 graduated flasks of 100 c.c. each, 3 of 50 c.c., and 3 of 25 c.c. Several liter flasks will also be found useful.

Erlenmeyer flasks are of thin glass like beakers and hence are very fragile, but are suited for boiling over gauze or asbestos or for the boiling water-bath. It is well to have 2 of 1000 c.c. capacity, and half a dozen smaller ones of capacity 250 c.c., 150 c.c. or smaller.

Beakers.—Beakers break easily, and it is just as well to provide no more than absolutely necessary for the amount of work re-

quired. A dozen of about 250 c.c. capacity (8 oz.) and half a dozen smaller ones, including several holding 50-100 c.c., will be needed. They should be lipped.

Burettes.—Half a dozen burettes of 25 c.c. capacity of the Schellbach variety with glass stop-cocks, and one or two of 50 c.c. capacity graduated in tenths will be needed. Burette clamps which screw into the shelf should be also provided.

Bottles and Jars.—Urine should not be allowed to stand in graduated ware, but after measurement should be poured into bottles or jars. Caustic soda bottles with a wide mouth make good urine receptacles after being thoroughly cleaned and rinsed with hot water. In addition it is well to have a dozen one pint fruit jars with tops for use in cases where urine mixtures are to stand for a period of time, as in Folin's method for uric acid.

Pipettes.—Graduated pipettes are serviceable and should include 5 c.c., 10 c.c., and 25 c.c., several of each. In addition there will be needed several curved nipple pipettes graduated to 1 c.c. and a dozen medicine droppers. For microscopical use, however, the author prefers glass tubing of the smallest sizes filed and carefully broken. These can be kept clean at all times and free from the impurities, etc., of rubber.

Funnels.—Glass funnels for purposes of filtration should be of two sizes, one size about 40 c.c. (1 oz.), and the larger about 240 c.c. (8 ounce) capacity. The long stems should be filed and broken off evenly. About a dozen funnels of these two sizes will usually suffice. Very small funnels, 5 or 10 c.c. capacity, are serviceable for use in burettes, being set in the top of a burette so that the latter may be filled without spilling from the bottle.

Wash Bottles.—Several will be needed, two of 500 c.c. capacity and two small ones of about 60 c.c. capacity.

Special Apparatus.—It is desirable to keep on hand several each of the following pieces of special apparatus: Einhorn's saccharimeter; Esbach's albuminimeter; Doremus' or Hinds' ureometer; Aufrecht's albuminimeter; Purdy's percentage tubes; the instrument known as the horismascope; Lohnstein's saccharimeter (to be imported); Mitchell's special centrifugal tubes (E. H. Sargent & Co., Chicago); conical glasses and wine-glasses; urino-

meter cylinders (the larger the better, those holding 120 c.c. preferred if they can be found); Squibb's urinometers; glass tray for staining; cover glasses; glass slides; square ground glass covers for conical sediment glasses; watch-glasses in nests of six; wire gauzes about 4 in. by 4, with and without asbestos centers; metallic filter- and burette-stands (1 or 2); one copper water-bath and rings; tripod for same; one or two triangles of wire for heating; one Sargent's improved Bunsen burner; one ordinary Bunsen; two metallic alcohol lamps; one chemical balance and weights (see Chapter III.); one centrifuge—hand, water or electric (see Chapter XXVI.); copper drying oven; desiccator; glass rods, several of small diameter—8 inches long—one or two long and thick—say, 18 inches by half an inch; Sahli's distilling apparatus (50 c.c. fractionating flask with projecting arm and test-tube); casseroles (250 c.c. and 500 c.c. capacity); porcelain evaporating dishes (250 and 500 c.c.); separatory funnels; Sohxlet's extraction apparatus; melting-point tubes; centigrade chemical thermometer; Folin's urea apparatus (Fig. 63); Folin's ammonia apparatus; Folin's improved absorption tube; apparatus for Kjeldahl nitrogen process; Bausch and Lomb polariscope; porcelain crucible, 25 c.c.; platinum crucible, 20 grammes; small platinum dish; platinum foil; platinum wire in glass rod handle; one Bausch and Lomb microscope with $^{3}/_{4}$ inch eye piece, $^{1}/_{2}$ and $^{1}/_{6}$ inch objectives; one $^{1}/_{12}$ inch oil immersion lens; four pegged test-tube racks, 2 holding 10 or 12 tubes and 2 holding 6 tubes each; one circular wire test-tube holder for a number of tubes; six burette holders with screw end; filter paper, No. 500, E. H. Sargent & Co. (4 in. and 8 in.); filter paper of known ash-weight, Schleicher and Schüll; a gross of test-tubes of three sizes, small, medium, and large, the small ones being long and narrow; half a dozen test-tube brushes, some with sponge ends; wooden test-tube holders, litmus paper (blue) 4 sheets cut into slips and kept in stoppered bottle.

Reagents.—These are either in solid or in liquid form. Those in solid form for general use are to be provided in amount, one pound each, and chemically pure, unless otherwise specified.

Acids.—Boric, citric, picric, tartaric, oxalic (8 oz. Merck's

guaranteed reagent), Schuchardt's purest boric acid crystals.

Ammonium Compounds.—Chloride, carbonate, sulphate, sulphocyanate, the sulphate in five-pound cartons.

Barium Compounds.—Chloride, oxide.

Copper Sulphate.—Merck's guaranteed reagent in 1 oz. bottles or more.

Iron.—Ferric chloride, Fe_2Cl_6, in lumps.

Lead Acetate.—Neutral and basic, colorless crystals.

Magnesium Sulphate.

Mercuric Chloride.—In one ounce bottles.

Mercuric Nitrate.—(See Millon's reagent.)



FIG. 63.—Folin's Urea Apparatus.

Phenylhydrazine Hydrochloride.—In one-ounce quantities.

Phenylhydrazine Mixture.—Grind up in a mortar 10 grammes of phenylhydrazine hydrochloride and 20 grammes sodium acetate, or any desired amount, in these proportions.

Potassium Compounds.—Chlorate, chromate, dichromate, ferrocyanide, hydroxide, iodide, permanganate; the hydroxide in stick form, one pound each of the ordinary and "by alcohol." The iodide in smaller amount (four ounces). The permanganate must be strictly pure, not commercial.

Potassium and Sodium Tartrate.—Rochelle salt.

Silver Nitrate.—In one-ounce bottles.

Sodium Compounds.—Sodium hydroxide by alcohol, half a dozen pounds in one pound bottles, separately; sodium acetate, carbonate, bicarbonate, chloride; sodium nitrite in one-ounce bottles; sodium nitroprussiate in one-ounce bottles.

Uranium.—Merck's reagents, acetate and nitrate (need not be sodium free) in four-ounce bottles.

Zinc Acetate and Chloride.—In small packages, a few ounces each.

Rarer Chemicals.—These must usually be procured of E. H. Sargent & Co., 125 West Lake Street, Chicago, or else imported.

Benzaldehyde (dimethylamino), alpha-naphthol diphenylamine, naphtho-resorcine, para-amino-acetophenone, etc., etc.

Liquid Reagents.—Large supplies of these may be kept in large acid bottles, *i. e.*, those used for 6 lbs. of hydrochloric acid, etc.

Liquid Reagents in Large Supply:—

Acetic Acid, glacial, c. p., 5 lbs.

Ammonia water, c. p., sp. gr. 0.90, 4 lbs.

Ammonium sulphate, 10 per cent. solution, 200 grammes of the salt to 1800 c.c. distilled water.

Ammonium sulpho-cyanate to be standardized for Luetke's reagent. (See below.) About 7.6 grammes in a little less than one liter of water.

Chloroform, Merck, c. p., 5 lbs.

Folin-Shaffer Reagent (for Uric Acid).—500 grammes of ammonium sulphate to 650 c.c. of distilled water to which 5 grammes of uranium acetate and 6 c.c. of glacial acetic acid are added with distilled water to make one liter.

Haines' Sugar Test Liquid.—48 grammes of Merck's reagent cupric sulphate to 360 c.c. of distilled water, 360 c.c., c. p., glycerin (Merck) (one pound), and 3600 c.c. liquor potassæ. (Liquor potassæ, see below.)

Hydrochloric Acid.—Baker and Adamson's c. p., sp. gr. 1.19, 6 lbs.

Nitric Acid, 25 Per cent.—528 c.c. of the chemically pure, sp. gr. 1.42, to 2250 c.c. distilled water (for use in Luetke's reagent).

Potassium Hydroxide Solution.—Liquor potassæ for use in Haines' solution—100 grammes potassium hydroxide to 1900 c.c.

distilled water. "Potassium hydroxide, Merck, reagent, purest" is the best, but the ordinary "c. p. by alcohol" usually suffices.

Sodium Acetate.—For use in the determination of phosphoric anhydride—200 grammes of sodium acetate c. p. and 200 c.c. of 30 per cent. acetic acid, in distilled water sufficient to dissolve and the whole diluted to make 2000 c.c. (Any convenient multiples of 10, 100 and 1000 may be used.)

Sodium Hydroxide.—For use in determining urea by the Doremus method. Dissolve the contents of two one pound bottles of c. p. sodium hydroxide in sticks in 2270 c.c. of distilled water, stirring so as not to allow the mass to cake and bearing in mind that the solution is very hot. (One pound to 1135 c.c. water or any convenient multiples.)

Sulphuric Acid.—Baker and Adamson's c. p. acid, sp. gr. 1.84, 9 pound bottle.

Uranium Nitrate.—For use in determining P_2O_5 in urine; dissolve 70.922 grammes of Merck's guaranteed reagent uranyl nitrate in distilled water to make 2000 c.c. Should be used up in a few months' time. 35.461 grammes in water to make a liter if smaller quantity is desired, or any convenient multiples.

Liquid Reagents in Smaller Quantity.—Certain reagents should be kept in small quantity and renewed frequently; others as, *e. g.*, volatile liquids, are likely to evaporate if kept too long, and a number are rarely used, hence are not needed in large amount.

Alcohol, U. S. P.—92.3% by weight, one pint.

Alcohol, 95%.—One pint, Merck's reagent.

Alcohol Absolute.—Merck's reagent, one pint.

Alcohol, Dilute, U. S. P.—41.5% weight, one pint.

Alcohol Amylic.—Merck's reagent, one pint.

Ammonium Chloride.—About 500 c.c. of a saturated solution of the chemically pure compound.

Acetic Acid, 50%.—Dilute 500 c.c. of glacial acid with the same volume of distilled water. Used in testing for albumin.

Aufrecht's Solution:—(See Chapter on Albumin).

Barium Chloride Mixture.—Hydrochloric acid (1.19), 24 c.c.; barium chloride c. p., 120 grammes; distilled water, 480 c.c. Used for the test for preformed sulphates.

Barium Chloride Solution, 10%.—100 grammes of the pure chloride and 900 c.c. water.

Burfoed's Solution.—Dissolve 1 gramme of neutral cupric acetate (Normal Merck) in pure crystals in 15 c.c. of water, and add to 200 c.c. of it 5 c.c. of 38 per cent, acetic acid.

Baryta Mixture.—Mix one volume of saturated barium nitrate solution with two of a saturated barium hydroxide solution.

Benzaldehyde.—The solution for testing the urobilinogen substances is made by dissolving 20 grammes of dimethylamino-benzaldehyde, para, in 1000 c.c. of dilute hydrochloric acid containing 150 c.c. of the acid per liter.

Benzoyl Chloride.—One pound, c. p.

Bial's Reagent.—Used as a test for pentoses. 500 c.c. of 30 per cent. hydrochloric acid (250 c.c. nearly of acid, sp. gr. 1.19, and 750 c.c. water), 1 gramme of orcin, and 25 drops of a 10 per cent. solution of ferric chloride.

Bromine.—One pound. (Larkin-Sheffer, St. Louis, Mo., if obtainable, or Mallinckrodt's.)

Bromine.—Rice's solution (E. H. Sargent & Co.) No. 1—100 grammes sodium hydroxide in 250 c.c. water. No. 2—1 part bromine and 1 part potassium bromide by weight to 8 of water. Mix equal parts (5 c.c.) and add 10 c.c. of water to the mixture to make the hypobromite solution for urea.

Calcium Hypochlorite.—Obtained by saturating water with commercial "chloride of lime;" sold in small metallic boxes; used as oxidizer in indican test.

Copper Sulphate.—For Fehling's solution 34.67 grammes of pure crystalline cupric sulphate dissolved in distilled water to measure 500 c.c. at 25° C., and kept in a rubber stoppered bottle. Use Merck's reagent. Also for the biuret test, dissolve 2 grammes c. p. in 98 c.c. distilled water.

Diazo Reagent of Ehrlich.—No. 1—Dissolve 0.5 gramme of c. p. sodium nitrite in 99 c.c. of distilled water and make up fresh in a few weeks. No. 2—Dissolve 5 grammes of sulphanilic acid, Merck's reagent, and 50 c.c. strong hydrochloric acid in water to make 1000 c.c. For use mix 1 and 2 in the proportion of 1 part by volume of No. 1 to 50 or 100 parts of No. 2.

Ether.—Merck's reagent, one pint.

Esbach's Solution.—Dissolve 20 grammes c. p. citric acid and 10 grammes c. p. picric acid in distilled water to make 1000 c.c.

Ferric Chloride.—20 grammes to 80 c.c. distilled water; also 5 grammes to 95 c.c.

Ferric Alum.—Dissolve 10 grammes of the salt in 90 c.c. of distilled water in the cold. (Grind up in mortar.)

Fehling's Solution.—500 c.c. each of the cupric sulphate and Rochelle salt solutions. Mix equal parts before using.

Formaldehyde.—Formaldehyde, Merck, in pound bottles. Highest purity. Used in Malfutti's ammonia determination.

Guaiac.—U. S. P. tincture freshly prepared, one pint or less. Dissolve 0.5 gramme guaiac in 30 c.c. of 95 per cent. alcohol.

Glycerin.—Merck's reagent, one pound, or in pound bottles and desired quantity.

Hydrogen Dioxide.—U. S. P., in small quantities, few ounces at a time.

Iodine.—Tincture U. S. P. (4 oz.), also in

Lugol's Solution.—5 grammes iodine, 10 grammes potassium iodide in water to make 100 c.c.; used for staining urinary sediments.

Lead Acetate.—Dissolve 20 grammes neutral lead acetate in colorless crystals in 80 c.c. of boiled and cooled distilled water. Solution must be colorless and kept from air. Used in Mitchell's indican test.

Lipliawsky's Reagent.—The Arnold-Lipliawsky solutions are as follows: No. 1—1 gramme c. p. potassium nitrite in 99 c.c. distilled water; No. 2—1 gramme of para-amino-acetophenon in 100 c.c. distilled water and about 2 c.c. of strong hydrochloric acid, drop by drop, to decolorize. Mix the two just before using.

Magnesia Mixture.—Dissolve 100 grammes each of c. p. magnesium sulphate and ammonium chloride in 800 c.c. of water containing 100 c.c. of strong ammonia water. Used for precipitating alkaline phosphates.

Millon's Reagent.—Digest 10 grammes of mercury in 14 c.c. of pure nitric acid and dilute the solution with twice its volume of water.

Moreigne's Reagent. Mix 20 grammes sodium tungstate c. p., 10 grammes (about 9 c.c.) phosphoric acid (sp. gr. 1.13) with 100 c.c. of distilled water, boil for 20 minutes, fill up to original bulk with water and add hydrochloric acid drop by drop until acid.

Nitric Acid.—Chemically pure, colorless, of specific gravity 1.42 in one pound bottles; rejected when yellow or else used when only traces of nitrous are needed.

Nitrous Acid.—Yellow fuming acid in one pound bottles or yellow nitric acid.

Nylander's Solution.—Digest two grammes of bismuth subnitrate and four grammes of Rochelle salt in 100 c.c. of a 10 per cent. solution of potassium hydroxide; cool and filter.

Obermayer's Solution.—Dissolve from 2 to 4 grammes of ferric chloride, Fe_2Cl_6, in 1000 c.c. of strong hydrochloric acid, sp. gr. 1.19. Askenstedt uses 3 grammes for his indican test liquid.

Phenolphthalein.—Dissolve 0.5 gramme of phenolphthalein in 95 per cent. alcohol to make 100 c.c.; another formula: 1 gramme in 100 c.c. 85 per cent. alcohol. Use Merck's reagent.

Purdy-Haines Solution.—Dissolve 8.314 grammes Merck's reagent cupric sulphate, 25 grammes Merck's reagent, potassium hydroxide, 40 grammes Merck's reagent glycerin (32 c.c.), and 350 grammes strong ammonia water (315 c.c.) in water to make a liter. Used for the quantitative determination of sugar.

Oxalic Acid, Decinormal.—Dissolve 6.225 grammes Merck's reagent in water to make 1000 c.c. at 25° C. (77° F.). Keep in an amber bottle and make up fresh every month. Used for standardizing potassium permanganate and sodium hydroxide solutions.

Potassium Ferrocyanide.—Dissolve 100 grammes of potassium ferrocyanide, Merck's reagent, in water to make 1000 c.c.

Potassium Chromate.—As an indicator saturate 100 c.c. of distilled water with the pure salt. 1 part to 2 of water.

Potassium Chlorate.—A four per cent. solution for work in ethereal sulphates is made by dissolving 40 grammes in 960 c.c. of water.

Potassium Permanganate.—The twentieth normal solution is made by boiling 1.58 grammes Merck's reagent (free from sulphuric acid) in water to make about a liter, cooling and titrating with decinormal oxalic acid, then diluting until 20 c.c. of it correspond to 10 c.c. of the oxalic acid solution diluted with 90 c.c. water with addition of 15 c.c. strong sulphuric acid.

Potassium and Sodium Tartrate; Rochelle Salt.—Dissolve 173 grammes of Rochelle salt and 75 grammes of potassium hydroxide in water to measure 500 c.c. Use Merck's reagents for Fehling's solutions.

Sodium Chloride.—Saturate 1000 c.c. of distilled water with c. p. sodium chloride. Do not use table salt.

Sewilanoff's Reagent.—Dissolve 0.05 gramme of resorcin in 100 c.c. of dilute hydrochloric acid (acid one part, water two parts).

Sodium Hydroxide, Decinormal.—Dissolve 6 grammes of Merck's reagent purified by alcohol in water to make about one liter and standardize with the decinormal oxalic acid solution, phenolphthalein indicator.

Spiegler's Reagent.—Dissolve 20 grammes tartaric acid, Merck's reagent, 40 grammes mercuric chloride (bichloride Merck, reagent), 100 grammes (80 c.c.) Merck's reagent glycerine in distilled water 1000 c.c.. (To make 1000 probably meant.)

Silver Nitrate Solution, Luetke.—For determination of chlorides dissolve 17.50 grammes of c. p. silver nitrate in 900 c.c. of 25 per cent. nitric acid, add to it 50 c.c. of a 10 per cent. ferric alum solution and dilute with distilled water to make a liter. Ammonium sulphocyanate as indicator.

Silver Nitrate Solution, Mohr.—Dissolve 29.06 grammes of pure silver nitrate in water to make one liter. Potassium chromate, saturated solution, as indicator.

Zinc Acetate.—For urobilin test dissolve 10 grammes zinc acetate, pure, in 30 grammes ammonia water (27 c.c.), add to this 80 grammes of 90 per cent. alcohol, and 20 grammes of acetic ether. Filter.

Benedict's Reagent for Sugar.—Copper sulphate, pure crystals, 8.65 grammes; sodium citrate, 86.50 grammes; sodium carbonate anhydrous, 50 grammes; distilled water to make 500 c.c. Dissolve the sodium compounds in 300 c.c. of water by aid of heat, filter, and add enough water to make 425 c.c. Dissolve the copper salt in 50 c.c. of water, filter and add water to make 75 c.c.. Mix by pouring the copper solution into the solution of sodium salts, stirring constantly. (See also page 337.)

Russo's Methylene-Blue Solution.—Dissolve one gramme of methylene-blue in 1000 c.c. of water, add 4 or 5 drops of 4 or 5 c.c. of urine, shake and an emerald-green color indicates a positive reaction; a blue color is negative. (The reaction has about the same value as the diazo in typhoid and tuberculosis. See Diazo Reaction.)

THERMOMETRIC EQUIVALENTS.

To convert Centigrade to Fahrenheit multiply by 1.8 and add 32. To convert Fahrenheit to Centigrade subtract 32 and multiply by 5/9.

4°	C. =	39.2°	F.
25°	C. =	77°	F.
37°	C. =	98.6°	F.
50°	C. =	122°	F.
60°	C. =	140°	F.
70°	C. =	158°	F.
80°	C. =	176°	F.
90°	C. =	194°	F.
100°	C. =	212°	F.

LIBRARY FOR COLLATERAL READING.

The worker in urine analysis should provide himself with a number of books for reference and collateral reading. The author heartily recommends the following: Simon's Chemistry, ninth edition, or later, if any; Bartley's Clinical Chemistry; Holland's Chemistry, latest edition; Emerson's Clinical Diagnosis, an invaluable work; C. E. Simon's Clinical Diagnosis; Hawk's Physiological Chemistry; Wood's Diagnostic Methods; Webster's Clinical Diagnosis; Croftan's Clinical Urinology; Sahli's work on Diagnosis; Saxe's Urine Analysis, and Dixon Mann's Clinical Diagnosis. Among periodicals Stern's *Archives of Diagnosis* contains abstracts of the new discoveries and methods of urinology, and should be consulted frequently.

Notes and observations coming up from time to time may be jotted down by the physician on the space below left blank, as, *e. g.*, the following in Stern's *Archives* for July, 1911: "Hematuria has great prognostic significane in eclampsia. Among thirty-six cases at Guy's Hospital ten had hematuria, and 6 died. Toxemia of pregnancy is very frequently associated with concealed accidental hemorrhage."

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